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(54) Title: REVERSIBLE CYSTEINE PROTEASE INHIBITORS		
(57) Abstract		
<p>A reversible cysteine protease inhibitor comprising two N-substituents linked via an ethylenediamine or a substituted ethylenediamine, wherein the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM, and wherein said N-substituents are selected from the group consisting of acyl, acylpeptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl.</p>		

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REVERSIBLE CYSTEINE PROTEASE INHIBITORS

FIELD OF THE INVENTION

The invention relates to novel reversible protease inhibitors. The inhibitors are selective for cysteine proteases.

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BACKGROUND OF THE INVENTION

Cysteine or thiol proteases contain a cysteine residue at the active site responsible for proteolysis. Since cysteine proteases have been implicated in a number of diseases, including arthritis, muscular dystrophy, inflammation, tumor invasion, glomerulonephritis, malaria, and other parasite-borne infections, methods for selectively and irreversibly inactivating them provide opportunities for new drug candidates. See, for example, Esser, R.E. et al., *Arthritis & Rheumatism* (1994) 37, 236; Meijers, M.H.M. et al., *Agents Actions* (1993), 39 (Special Conference Issue), C219; Machleidt, W. et al, *Fibrinolysis* (1992), 6 Suppl. 4, 125; Sloane, B.F. et al., *Biomed. Biochim. Acta* (1991), 50, 549; Duffy, M.J., *Clin. Exp. Metastasis* (1992), 10, 145; Rosenthal, P.J., Wollish, W.S., Palmer, J.T., Rasnick, D., *J. Clin. Investigations* (1991), 88, 1467; Baricos, W.H. et al, *Arch. Biochem. Biophys.* (1991), 288, 468; Thornberry, N.A. et al., *Nature* (1992), 356, 768.

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Low molecular weight inhibitors of cysteine proteases have been described by Rich, *Proteinase Inhibitors* (Chapter 4, "Inhibitors of Cysteine Proteinases"), Elsevier Science Publishers (1986). Such inhibitors include peptide aldehydes, which form hemithioacetals with the cysteine of the protease active site. See, for instance, Cheng, H., Keitz, P., and Jones, J.B., *J. Org. Chem.* (1994), 59, 7671. The disadvantage of aldehydes is their in vivo and chemical instabilities.

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Methods for selectively and irreversibly inhibiting cysteine proteases have relied upon alkylation by peptide α -fluoromethyl ketones (RASNICK, D., *Anal. Biochem.* (1985), 149, 416), diazomethyl-ketones (KIRSCHKE, H., SHAW, E. *Biochem. Biophys. Res. Commun.* (1981), 101, 454), acyloxymethyl ketones (KRANTZ, A. et al., *Biochemistry*, (1991), 30, 4678; KRANTZ, A. et al., U.S. Patent 5,055,451, issued October 8, 1991), and 5 ketosulfonium salts (WALKER, B., SHAW, E., *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, (1985), 44, 1433).

Other families of cysteine protease inhibitors include epoxysuccinyl peptides, including E-64 and its analogs (HANADA, K. et al., *Agric. Biol. Chem.* (1978), 42, 523; SUMIYA, S. 10 et al., *Chem. Pharm. Bull.* ((1992), 40, 299 GOUR-SALIN, B.J. et al., *J. Med. Chem.*, (1993), 36, 720), α -dicarbonyl compounds, reviewed by MEHDI, S., *Bioorganic Chemistry*, (1993), 21, 249, and N-peptidyl-O-acyl hydroxamates (BROMME, D., NEUMANN, U., KIRSCHKE, H., DEMUTH, H-U., *Biochim. Biophys. Acta*, (1993), 1202, 271. An additional summary of methods for reversibly and irreversibly inhibiting cysteine 15 proteases has recently been compiled; see SHAW, E., *Advances in Enzymology and Related Areas of Molecular Biology* (1990), 63, 271.

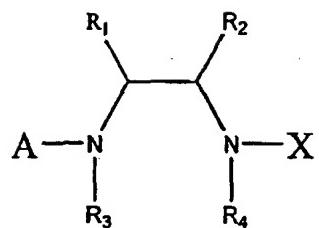
SUMMARY OF THE INVENTION

It is an object of the present invention to provide novel cysteine protease inhibitors that function reversibly, resulting in tight binding (low dissociation constants) between 20 inhibitor and target enzyme. It is a further object to provide these novel cysteine protease inhibitors for use in a variety of therapeutic applications.

In accordance with the foregoing objects, one embodiment of the present invention provides reversible cysteine protease inhibitors comprising two N-substituents linked via an ethylenediamine or a substituted ethylenediamine, wherein the dissociation 25 constant for inhibition, K_i , of a protease with the inhibitor, is no greater than about 100 μM , and wherein said N-substituents are selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl.

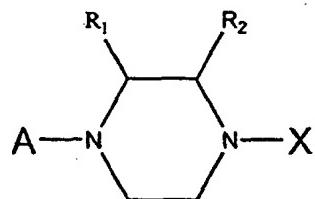
30 Also provided are reversible cysteine protease inhibitor having the formula comprising:

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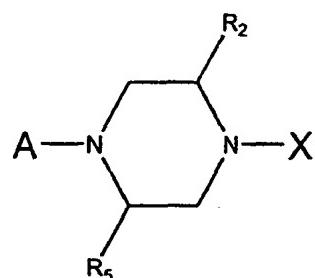


In this aspect, A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl. R₁ is either hydrogen or an amino acid side chain, and R₂ is either hydrogen or an amino acid side chain. However, either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen. R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene. Additionally, the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μ M.

10 Also provided are reversible cysteine protease inhibitors having the formula comprising:

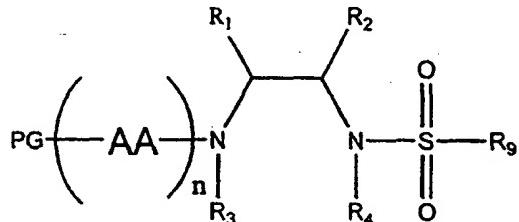


Further provided are reversible cysteine protease inhibitors having the formula comprising:



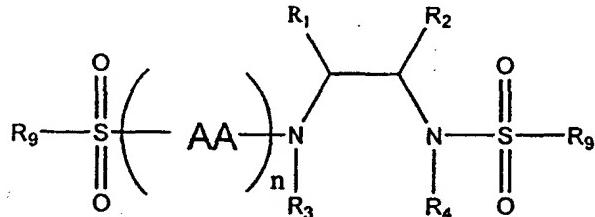
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Additionally provided are reversible cysteine protease inhibitors having the formula comprising:



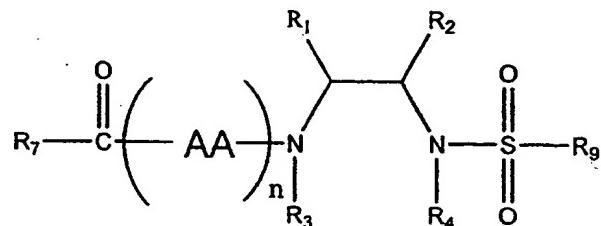
In this aspect, n is from 1 to 10, PG is a protecting group, and AA is an amino acid. R₁ is either hydrogen or an amino acid side chain, and R₂ is either hydrogen or an amino acid side chain. However, either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen. R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene. -SO₂R₉ is a sulfonyl moiety. Additionally, the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

Further provided are reversible cysteine protease inhibitor having a formula comprising:



In this aspect, n is from 1 to 10 and AA is an amino acid. R₁ is either hydrogen or an amino acid side chain, and R₂ is either hydrogen or an amino acid side chain. However, either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen. R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene. -SO₂R₉ is a sulfonyl moiety. Additionally, the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

An additional aspect of the invention provides reversible cysteine protease inhibitors having a formula comprising:



In this aspect, n is from 1 to 10, PG is a protecting group and AA is an amino acid. R₁ is either hydrogen or an amino acid side chain, and R₂ is either hydrogen or an amino acid side chain. However, either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen. R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene. -SO₂R₉ is a sulfonyl moiety. Additionally, the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

A further aspect of the invention provides methods of inhibiting a cysteine protease inhibitor comprising reversibly binding a cysteine protease inhibitor of the invention to a cysteine protease.

An additional aspect provides cysteine proteases inhibited by the cysteine protease inhibitors of the invention.

Further, the invention provides methods of treating cysteine protease associated disorders comprising administering to a patient a therapeutically effective dose of cysteine protease inhibitor of the invention, and pharmaceutical compositions comprising the cysteine protease inhibitors of the invention.

Additionally, the invention provides methods of detecting a cysteine protease in a sample comprising assaying the sample for protease activity using a protease substrate. The sample is then assayed for protease activity in the presence of a known concentration of a cysteine protease inhibitor of the invention, and determining the amount of cysteine protease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, 1O, 1P, 1Q, 1R, 1S,
1T, 1U, 1V, 1W, 1X, 1Y, 1Z, 1AA, 1BB, 1CC, and 1DD depict the structures of some of
5 the cysteine protease inhibitors of the invention. The structures, abbreviated names,
and kinetic data are shown, as described more fully in the Examples. The kinetic data
are in micromolar units.

Figure 2 depicts a schematic synthesis for cysteine protease inhibitors with R₂
substituent groups on ethylenediamine moieties. 1. Carbonyldiimidazole, NH₃/THF; 2.
10 HCl/dioxane, CH₂Cl₂/ether; 3. R₉SO₂Cl, triethyl amine (TEA), THF; 4. LAH or suitable
reducing agent, THF, reflux; 5. HCl/dioxane, CH₂Cl₂/ether; 6. PG-AA,-OH, NMM, IBCF,
THF at -10°C.

Figure 3 depicts a schematic synthesis for cysteine protease inhibitors with R₁
substituent groups on ethylenediamine moieties. 1. LiAlH₄ (LAH) or suitable reducing
agent, THF, reflux; 2. HCl/dioxane, CH₂Cl₂/ether; 3. R₉SO₂Cl, TEA, THF, -10°C; 4.
15 H₂/Pd/C or suitable catalyst, HCl/dioxane, EtOH; 5. PG-AA,-OH, 4-methyl morpholine
(NMM); isobutyl chloroformate (IBCF), THF, -10°C.

Figure 4 depicts a schematic synthesis for cysteine protease inhibitors with R₂ and R₅
substituent groups on piperazine moieties. 1. Activation of acid: pyr, TC, THF, -10°C;
2. bis(trimethylsilyl)acetamide (BSA), TEA, THF; 3. HBr/AcOH; 4. TEA, EtOH; 5. LAH
20 or suitable reducing agent, THF, reflux; 6. HCl/dioxane, CH₂Cl₂/ether; 7. R₉SO₂Cl,
TEA, THF; 8. H₂/Pd/C or suitable catalyst, EtOH; 9. pyr, TC, THF, -10°C; 10.
compound 2, BSA, TEA, THF.

Figure 5 depicts a schematic synthesis for cysteine protease inhibitors with R₁ and R₆
substituent groups on piperazine moieties. Step 1 is the activation of the acid: pyridine
25 (pyr), thionyl chloride (TC), THF, at -10°C; 2. bis (trimethylsilyl)acetamide (BSA), TEA,
THF; 3. HBr/AcOH; 4. TEA, EtOH; 5. LAH or suitable reducing agent, THF, reflux; 6.
HCl/dioxane, CH₂Cl₂/ether; 7. pyr, TC, THF; 8. compound 1, BSA, TEA, THF; 9.
H₂/Pd/C or suitable catalyst, HCl/dioxane, Et₂O; 10. R₉SO₂Cl, TEA, THF.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel cysteine protease inhibitors. It is believed that the enzyme is reversibly inhibited by means of interactions between the N-blocking groups and the R₁ or R₂ group of the inhibitor and the surface of the binding sites of the enzyme, via hydrogen bonding and hydrophobic interactions.

Generally, the inhibitors of the present invention inhibit cysteine proteases and do not inhibit serine, aspartyl, and zinc proteases. The mechanisms of serine protease action have been described by Walsh, C., in "Enzymatic Reaction Mechanisms" pp. 94-97, W.H. Freeman and Co., San Francisco, 1979. The serine at the active site reacts with the carbonyl of the substrate, forming a tetrahedral intermediate. The inhibitors of this invention have no carbonyl at the site of nucleophilic attack, and are not susceptible to attack by serine proteases.

However, in some embodiments, the protease inhibitors of the present invention may have activity against other types of proteases, such as serine, aspartyl or other metalloproteases, but to a lesser extent. In particular, the protease inhibitors of the invention may have activity against serine proteases, for example, they may have activity against chymotrypsin.

Cysteine proteases are a family of proteases that bear a thiol group at the active site. These proteases are found in bacteria, viruses, eukaryotic microorganisms, plants, and animals. Cysteine proteases may be generally classified as belonging to one of four or more distinct superfamilies. Examples of cysteine proteases that may be inhibited by the novel cysteine protease inhibitors of the present invention include, but are not limited to, the plant cysteine proteases such as papain, ficin, aleurain, oryzain and actinin; mammalian cysteine proteases such as cathepsins B, H, J, L, N, S, T, O, O₂ and C, (cathepsin C is also known as dipeptidyl peptidase I), interleukin converting enzyme (ICE), calcium-activated neutral proteases, calpain I and II; bleomycin hydrolase, viral cysteine proteases such as picornian 2A and 3C, aphthovirus endopeptidase, cardiovirus endopeptidase, comovirus endopeptidase, polyvirus endopeptidases I and II, adenovirus endopeptidase, the two endopeptidases from chestnut blight virus, togavirus cysteine endopeptidase, as well as cysteine proteases of the polio and rhinoviruses; and cysteine proteases known to be essential for

parasite lifecycles, such as the proteases from species of *Plasmodia*, *Entamoeba*, *Onchocera*, *Trypanosoma*, *Leishmania*, *Haemonchus*, *Dictyostelium*, *Therileria*, and *Schistosoma*, such as those associated with malaria (*P. falciparum*), trypanosomes (*T. cruzi*, the enzyme is also known as cruzain or cruzipain), murine *P. vinckeii*, and the *C. elegans* cysteine protease. For an extensive listing of cysteine proteases that may be inhibited by the cysteine protease inhibitors of the present invention, see Rawlings et al., Biochem. J. 290:205-218 (1993), hereby expressly incorporated by reference.

Accordingly, inhibitors of cysteine proteases are useful in a wide variety of applications. For example, the inhibitors of the present invention are used to quantify the amount of cysteine protease present in a sample, and thus are used in assays and diagnostic kits for the quantification of cysteine proteases in blood, lymph, saliva, or other tissue samples, in addition to bacterial, fungal, plant, yeast, viral or mammalian cell cultures. Thus in a preferred embodiment, the sample is assayed using a standard protease substrate. A known concentration of cysteine protease inhibitor is added, and allowed to bind to a particular cysteine protease present. The protease assay is then rerun, and the loss of activity is correlated to cysteine protease activity using techniques well known to those skilled in the art.

Thus, methods of inhibiting a protease are provided, wherein the cysteine protease inhibitors of the invention may be added to a sample of cysteine protease, to form a cysteine protease/cysteine protease inhibitor complex.

Additionally, the cysteine protease inhibitors are also useful to remove, identify or inhibit contaminating cysteine proteases in a sample. For example, the cysteine protease inhibitors of the present invention are added to samples where proteolytic degradation by contaminating cysteine proteases is undesirable. Alternatively, the cysteine protease inhibitors of the present invention may be bound to a chromatographic support, using techniques well known in the art, to form an affinity chromatography column. A sample containing an undesirable cysteine protease is run through the column to remove the protease. Alternatively, the same methods may be used to identify new proteases.

In a preferred embodiment, the cysteine protease inhibitors are useful for inhibiting cysteine proteases implicated in a number of diseases. In particular, cathepsins B, L,

- and S, cruzain, calpains I and II, and interleukin 1 β converting enzyme are inhibited. These enzymes are examples of lysosomal cysteine proteases implicated in a wide spectrum of diseases characterized by tissue degradation. Such diseases include, but are not limited to, arthritis, muscular dystrophy, inflammation, tumor invasion,
- 5 glomerulonephritis, parasite-borne infections, Alzheimer's disease, periodontal disease, and cancer metastasis. For example, mammalian lysosomal thiol proteases play an important role in intracellular degradation of proteins and in the processing of some peptide hormones. Enzymes similar to cathepsins B and L are released from tumors and may be involved in tumor metastasis. Cathepsin L is present in diseased
- 10 human synovial fluid and transformed tissues. Similarly, the release of cathepsin B and other lysosomal proteases from polymorphonuclear granulocytes and macrophages is observed in trauma and inflammation.
- The cysteine protease inhibitors also find application in a multitude of other diseases, including, but not limited to, gingivitis, malaria, leishmaniasis, filariasis, osteoporosis
- 15 and osteoarthritis, and other bacterial and parasite-borne infections. The compounds also offer application in viral diseases, based on the approach of inhibiting proteases necessary for viral replication. For example, many picornaviruses including poliovirus, foot and mouth disease virus, and rhinovirus encode for cysteine proteases that are essential for cleavage of viral polyproteins.
- 20 Additionally, these compounds offer application in disorders involving interleukin-1 β converting enzyme (ICE), a cysteine protease responsible for processing interleukin 1 β ; for example, in the treatment of inflammation and immune based disorders of the lung, airways, central nervous system and surrounding membranes, eyes, ears, joints, bones, connective tissues, cardiovascular system including the pericardium,
- 25 gastrointestinal and urogenital systems, the skin and the mucosal membranes. These conditions include infectious diseases where active infection exists at any body site, such as meningitis and salpingitis; complications of infections including septic shock, disseminated intravascular coagulation, and/or adult respiratory distress syndrome; acute or chronic inflammation due to antigen, antibody and/or complement deposition;
- 30 inflammatory conditions including arthritis, cholangitis, colitis, encephalitis, endocarditis, glomerulonephritis, hepatitis, myocarditis, pancreatitis, pericarditis, reperfusion injury and vasculitis. Immune-based diseases include but are not limited to conditions involving T-cells and/or macrophages such as acute and delayed

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- hypersensitivity, graft rejection, and graft-versus-host disease; auto-immune diseases including Type I diabetes mellitus and multiple sclerosis. Bone and cartilage reabsorption as well as diseases resulting in excessive deposition of extracellular matrix such as interstitial pulmonary fibrosis, cirrhosis, systemic sclerosis, and keloid formation may also be treated with the inhibitors of the present invention. The inhibitors may also be useful in the treatment of certain tumors that produce IL 1 as an autocrine growth factor and in preventing the cachexia associated with certain tumors. Apoptosis and cell death are also associated with ICE and ICE-like activities and may be treated with the inhibitors of the present invention.
- 10 Furthermore, the cysteine protease inhibitors of the present invention find use in drug potentiation applications. For example, therapeutic agents such as antibiotics or antitumor drugs can be inactivated through proteolysis by endogenous cysteine proteases, thus rendering the administered drug less effective or inactive. For example, it has been shown that bleomycin, an antitumor drug, can be hydrolyzed by bleomycin hydrolase, a cysteine protease (see Sebti et al., Cancer Res. January 1991, pages 227-232). Accordingly, the cysteine protease inhibitors of the invention may be administered to a patient in conjunction with a therapeutic agent in order to potentiate or increase the activity of the drug. This co-administration may be by simultaneous administration, such as a mixture of the cysteine protease inhibitor and the drug, or by separate simultaneous or sequential administration.
- 15 In addition, cysteine protease inhibitors have been shown to inhibit the growth of bacteria, particularly human pathogenic bacteria (see Bjorck et al., Nature 337:385 (1989)). Accordingly, the cysteine protease inhibitors of the present invention may be used as antibacterial agents to retard or inhibit the growth of certain bacteria.
- 20 25 The cysteine protease inhibitors of the invention also find use as agents to reduce the damage of bacterial cysteine proteases to host organisms. For example, *staphylococcus* produces a very active extracellular cysteine protease which degrades insoluble elastin, possibly contributing to the connective tissue destruction seen in bacterial infections such as septicemia, septic arthritis and otitis. See Potempa et al., J. Biol. Chem. 263(6):2664-2667 (1988). Accordingly, the cysteine protease inhibitors of the invention may be used to treat bacterial infections to prevent tissue damage.
- 30

The present invention generally provides new peptide-based and peptidomimetic cysteine protease inhibitors for use as reversible cysteine protease inhibitors. By "cysteine protease inhibitor" herein is meant an inhibitor which inhibits cysteine proteases. In a preferred embodiment, the cysteine protease inhibitors are specific to cysteine proteases; that is, they do not inhibit other types of protease such as serine, aspartyl, or other metalloproteases. However, in alternative embodiments, the cysteine protease inhibitors of the invention may inhibit other types of proteases as well; for example, they may have activity against serine proteases.

By "reversible" herein is meant that the inhibitor binds non-covalently to the enzyme, and is to be distinguished from irreversible inhibition. See Walsh, Enzymatic Reaction Mechanisms, Freeman & Co., N.Y., 1979. "Reversible" in this context is a term understood by those skilled in the art. In addition, the reversible cysteine protease inhibitors are competitive inhibitors, that is, they compete with substrate in binding reversibly to the enzyme, with the binding of inhibitor and substrate being mutually exclusive. In addition, the stoichiometry of inhibition is 1:1; that is, a single inhibitor molecule is sufficient to inhibit a single enzyme molecule.

Unless otherwise stated, the following terms used in the specification and claims are defined for the purposes of this application and have the meanings given below:

- "Alkyl", as in alkyl, alkyloxy, alkylthio, alkylsulfonyl, alkylcarbamoyl, dialkylcarbamoyl, heteroarylalkyl, arylalkyl, and the like, means a straight or branched, saturated or unsaturated hydrocarbon radical having from 1 to 10 carbon atoms or the number of carbon atoms indicated (e.g., methyl, ethyl, prop-yl, isopropyl, butyl, sec-butyl, isobutyl, *tert*-butyl, vinyl, allyl, 1-propenyl, isopropenyl, 1-but enyl, 2-but enyl, 3-but enyl, 2-methylallyl, ethynyl, 1-propynyl, 2-propynyl, etc.).
- "Cycloalkyl", as in cycloalkyl and cycloalkylalkyl, means a saturated or unsaturated, monocyclic or polycyclic hydrocarbon radical containing 3 to 20 carbon atoms or the number of carbon atoms indicated, wherein the carbon atom with the free valence is a member of a non-aromatic ring, and any carbocyclic ketone and thiketone derivative thereof (e.g., the term cycloalkyl is meant to include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, bicyclo[2.2.2]octyl, 1,2,3,4-tetrahydro-1-naphthyl, oxocyclohexyl, dioxocyclohexyl, thiocyclohexyl, 9-fluorenyl, etc.).

"Heterocycloalkyl", as in heterocycloalkyl, heterocycloalkylalkanoylamino, heterocycloalkylcarbonyl, heterocycloalkylcarbonyl, and the like, means cycloalkyl as defined above wherein 1 to 5 of the indicated carbon atoms is replaced by a heteroatom chosen from N, O, S, P or As, wherein the atom with the free valence is a member of a non-aromatic ring, and any heterocyclic ketone, thiketone, sulfone or sulfoxide derivative thereof, (e.g., the term heterocycloalkyl is meant to include piperidyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, indolinyl, quinuclidinyl, morpholinyl, piperazinyl, *N*-methylpiperazinyl, piperadanyl, 4,4-dioxo-4-thiapiperidinyl, 1,2,3,4-tetrahydro-3-isoquinolyl, 2,4-diaza-3-oxo-7-thia-6-bicyclo[3.3.0]octyl, etc.).

Thus, hetero(C₆)cycloalkyl includes the radicals morpholinyl, piperazinyl, piperidinyl and the like.

"Aryl" means an aromatic monocyclic or polycyclic hydrocarbon radical containing 6 to 14 carbon atoms or the number of carbon atoms indicated and any carbocyclic ketone or thiketone derivative thereof, wherein the carbon atom with the free valence is a member of an aromatic ring, (e.g., aryl includes phenyl, naphthyl, anthracenyl, phenanthrenyl, 1,2,3,4-tetrahydro-5-naphthyl, 1-oxo-1,2-dihydro-5-naphthyl, 1-thioxo-1,2-dihydro-5-naphthyl, etc.). For the purposes of this application aryl includes heteroaryl. "Heteroaryl" means an aromatic monocyclic or polycyclic hydrocarbon radical containing overall from 5 to 14 atoms or the number of atoms indicated, wherein 1 to 5 of the indicated carbon atoms are replaced by a heteroatom chosen from N, O, S, P or As, wherein the atom with the free valence is a member of an aromatic ring, and any heterocyclic ketone and thiketone derivative thereof (e.g., the term heteroaryl is meant to include thienyl, furyl, pyrrolyl, pyrimidinyl, isoxazolyl, oxazolyl, indolyl, benzo[b]thienyl, isobenzofuranyl, purinyl, isoquinolyl, pterdanyl, pyrimidinyl, imidazolyl, pyridyl, pyrazolyl, pyrazinyl, 4-oxo-1,2-dihydro-1-naphthyl, 4-thioxo-1,2-dihydro-1-naphthyl, etc.). Thus, hetero(C₆)aryl includes the radicals pyridyl, pyrimidinyl, and the like.

"1,2-Phenylenedimethylene" means a divalent radical of the formula -CH₂C₆H₄CH₂-.

For example, the group R₁₃-Y-Z-X₃- in which Y is -N(R₁₄), Z is -CH(R₁₆)-, X₃ is carbonyl and R₁₄ together with R₁₆ forms 1,2-diphenylenedimethylene" is 1,2,3,4-tetrahydro-3-isoquinolylcarbonyl and substituted derivatives and individual stereoisomers and mixture of stereoisomers thereof. Substituted derivatives of the

1,2-phenylenedimethylene divalent radical may contain a hydroxy group on any carbon within the ring system or an oxo group on either of the unsaturated ring carbon atoms.

"Methylene" as in "(C₃₋₄)methylene" and "(C₃₋₇)methylene" mean a straight, saturated divalent radical having the number of carbon atoms indicated. For example,
5 "C₃₋₄)methylene" includes trimethylene -(CH₂)₃- and tetramethylene -(CH₂)₄-). Thus, the group R₁₃-Y-Z-X₃- in which Y is -N(R₁₄), Z is -CH(R₁₆)-, X₃ is carbonyl and R₁₄ together with R₁₆ forms trimethylene is 2-pyrrolidinylcarbonyl and the individual stereoisomers and mixtures of stereoisomers thereof. Substituted derivatives of the trimethylene and tetramethylene divalent radicals may contain a hydroxy group, or a
10 protected derivative thereof, or an oxo group on any of the ring carbon atoms. Suitable hydroxy protective groups are defined below.

"Oxa(C₃₋₇)methylene" and "aza(C₃₋₇)methylene" mean methylene as defined above wherein one of the indicated carbon atoms is replaced by an oxygen or nitrogen atom, respectively. For example, "oxa(C₅)methylene" includes 3-oxapentamethylene
15 (-CH₂CH₂OCH₂CH₂-) and 2-oxapentamethylene (-CH₂OCH₂CH₂CH₂-). Thus, -C(O)NR₁₀R₁₁, means the radical 4-morpholinylcarbonyl when R₁₀ and R₁₁, together form 3-oxapentamethylene and the radical 1-piperazinylcarbanoyl when R₁₀ and R₁₁, together form 3-azapentamethylene.

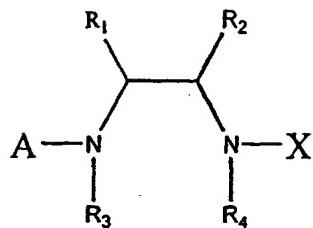
"Adjacent", as use in the phrase "R₁₆ together with an adjacent R₁₄", means that the
20 atoms to which the R₁₆ and R₁₄ groups are respectively attached are in turn attached to one another.

In the broadest embodiment, the cysteine protease inhibitors of the present invention comprise two N-substituents linked via an ethylenediamine or piperazine group.

Suitable N-substituents include, but are not limited to, acyl, alkyloxycarbonyl, sulfonyl,
25 sulfamoyl, peptidyl, carbamoyl, sulfinyl, aralkyl, or hydrogen, or combinations thereof, including, but not limited to, acyl peptidyl, alkyloxycarbonyl peptidyl, sulfonyl peptidyl, sulfamoyl peptidyl, carbamoyl peptidyl and sulfinyl peptidyl.

In a preferred embodiment, the reversible cysteine protease inhibitors of the present invention comprise compositions having the formula depicted in Formula 1:

Formula 1



In this embodiment, A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, carbamoyl, carbamoyl peptidyl, 5 sulfinyl, and sulfinyl peptidyl. R₁ and R₂ are hydrogen or an amino acid side, wherein only 1 of R₁ or R₂ is an amino acid side chain and the other one of R₁ or R₂ is hydrogen. R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene.

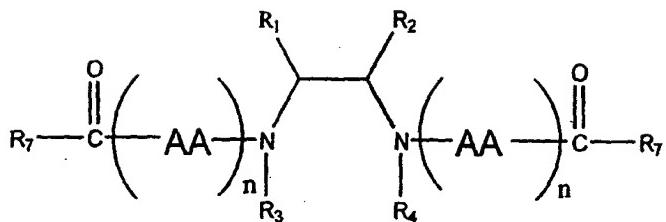
By "acyl" herein is meant a -COR₁ group. Suitable R₁ groups include, but not limited to, an alkyl (forming alkanoyl), a cycloalkyl (forming cycloalkylcarbonyl), a cycloalkylalkyl, a cycloalkylalkenyl, an aryl (forming aroyl), or an aralkyl. In such an instance, alkyl is preferably of 1 to 5 carbon atoms, especially ethyl. Cycloalkyl preferably is of 3 to 7 carbon atoms, preferably cyclopentyl or cyclohexyl. Cycloalkylalkyl or cycloalkylalkenyl preferably is of 3 to 7 carbon atoms in the cycloalkyl, particularly 5 or 6 carbon atoms, and of 1 to 5 carbon atoms, particularly 1 carbon atom, in the alkyl or alkylene moieties thereof. Heterocycloalkyl (forming heterocycloalkylcarbonyl) is also preferred, optionally substituted with a radical selected from hydroxy, alkyl, heterocycloalkyl, alkanoyl, alkyloxycarbonyl, arylalkyloxycarbonyl and heterocycloalkylcarbonyl. Aryl preferably is phenyl. Aralkyl preferably is phenylalkyl of 7 to 12 carbon atoms, particularly benzyl. The optional substituents of an aryl or aralkyl moiety preferably are one or two groups alkyl of 1 to 5 carbon atoms, alkoxy of 1 to 5 carbon atoms, halogen of atomic number of from 9 to 20, hydroxy and/or amino, preferably one or two groups methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, particularly one hydroxy, amino, chlorine, bromine, or fluorine, optionally in protected form where appropriate, nitro, alkyl or arylsulfonyl, or halogen-substituted alkyl of 1 to 5 carbon atoms, particularly 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96 101 106 111 116 121 126 131 136 141 146 151 156 161 166 171 176 181 186 191 196 201 206 211 216 221 226 231 236 241 246 251 256 261 266 271 276 281 286 291 296 301 306 311 316 321 326 331 336 341 346 351 356 361 366 371 376 381 386 391 396 401 406 411 416 421 426 431 436 441 446 451 456 461 466 471 476 481 486 491 496 501 506 511 516 521 526 531 536 541 546 551 556 561 566 571 576 581 586 591 596 601 606 611 616 621 626 631 636 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trifluoromethyl. Also included are perfluoro groups, such as perfluoro alkyl, aryl, and aralkyl.

- Particularly preferred in regards to R, are: (1) (C1-C5)alkyl, preferably ethyl; (2) (C3-C7)cycloalkyl, preferably cyclopentyl or cyclohexyl; (3) (C3-C7)cycloalkyl-(C1-C5)alkyl, especially (C5-C6)cycloalkyl-methyl; (4) (C3-C7)cycloalkyl-(C1-C5)alkenyl, especially (C5-C6)cycloalkyl-methylene; (5) phenyl; (6) (C7-C12)phenylalkyl, especially benzyl; (7) aryl or aralkyl substituted by one or two groups of C1-C5 alkyl, C1-C5 alkoxy, halogen, hydroxy or amino, with one or two groups of methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino being preferred and hydroxy, amino, chlorine, bromine or fluorine being particularly preferred; (11) aryl or aralkyl substituted by one or two groups of halogen-substituted C1-C5 alkyl, especially trifluoromethyl; nitro; sulfonyl; or arylsulfonyl, in protected form where appropriate; (12) perfluoro groups, such as perfluoro alkyl, aryl, and aralkyl. Most preferred R, acyl groups include phenyl and 4-morpholinyl.
- 15 Thus, preferred acyl groups are alkyloxycarbonylalkanoyl, arylalkanoyl, aroyl and alkanoyl groups (optionally substituted with a radical selected from carboxy, alkyloxycarbonyl and heterocycloalkylalkanoylamino), cycloalkylcarbonyl, heterocycloalkylcarbonyl (optionally substituted with a radical selected from hydroxy, alkyl, heterocycloalkyl, alkanoyl, alkyloxycarbonyl, arylalkyloxycarbonyl and heterocycloalkylcarbonyl), arylalkanoyl, and aroyl.
- 20 By "acyl peptidyl" or "peptidyl acyl" herein is meant a peptidyl group linked to an acyl group. It is to be understood that the peptidyl group is linked to one of the nitrogens of the ethylenediamine or piperazine moieties, and the acyl group is linked to the peptidyl group. Thus, the free terminal functionality of an acyl peptidyl group is the R₇ group.
- 25 Accordingly, the order of the functionalities differs depending on whether it is the A group or the X group which is the acyl peptidyl; the group is called an acyl peptidyl moiety when describing the A group and a peptidyl acyl moiety when describing the X group. Formula 2 shows a cysteine protease inhibitor with an acyl peptidyl group at the A and a peptidyl acyl group at the X position, wherein n is from 1 to 10.

-16-

Formula 2



As is described below, the choice of the peptidyl groups will depend on the protease to be inhibited.

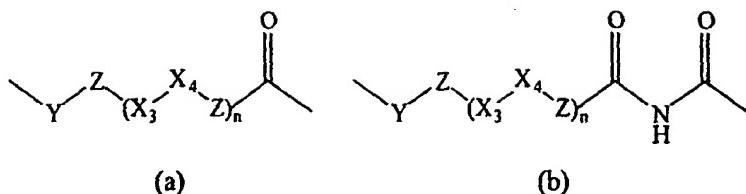
- By "peptidyl" herein is meant a peptide or peptidomimetic structure. In some 5 embodiments, peptidyl is a single amino acid; in other embodiments, the peptidyl group comprises at least two amino acids linked via a peptide bond or isostere. The peptidyl group may include up to about 10 amino acids, with 1 to 7 being preferred, although cysteine protease inhibitors are generally from about 1 to about 4 amino acids in length, since smaller inhibitors are usually desired in therapeutic applications.
- 10 The peptidyl group may comprise naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. "analogs". Thus "amino acid", "peptide residue", or "peptidyl" as used herein means both naturally occurring and synthetic amino acids, i.e. amino acid analogs. For example, homo-phenylalanine is considered an amino acid for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The term as used herein also refers to portions of an amino acid, such as an amino acid side chain. Thus, R₁, R₂, R₅, R₆ and 15 R₁₆, for example, may be amino acid side chains, which includes naturally occurring amino acid side chains as well as non-naturally occurring side chain analogs as described herein. The side chains may be in either the (R) or the (S) configuration. In 20 a preferred embodiment, the amino acids are in the (S) or L-configuration.

- If non-naturally occurring side chains are used, i.e. amino acid analogs, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradations. Such non-amino acid substituents will normally include, but are not limited to, an alkyl (optionally substituted with a radical selected from hydroxy, alkoxy, amino, 25 alkylamino, dialkylamino, uriedo, alkyluriedo, mercapto, alkylthio, carboxy, carbamoyl,

alkylcarbamoyl, dialkylcarbamoyl, alkylsulfinyl, alkylsulfonyl, guanidino, -P(O)(OR₁₂)₂, -OP(O)(OR₁₂)₂ or -OP(O)(R₁₂)₂, wherein each R₁₂ is independently hydrogen or alkyl, or a protected derivative thereof), cycloalkyl, cycloalkylalkyl, cycloalkylalkenyl, a group selected from aryl (including heteroaryl) and arylalkyl (including heteroarylalkyl and heteroarylalkenyl), (which group is optionally substituted at its aryl ring with one to three radicals selected from hydroxy, amino, guanidino, halo, optionally halo-substituted alkyl, alkyloxy and aryl, or a protected derivative thereof), alkoxy, cyano, carboxy, alkyloxycarbonyl, alkanoyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkyloxy(alkyl)carbamoyl, or aminoalkylcarbamoyl. In such an instance, alkyl is preferably of 1 to 5 carbon atoms, preferably branched, particularly isobutyl. Cycloalkyl preferably is of 3 to 7 carbon atoms, preferably cyclopentyl or cyclohexyl. Cycloalkylalkyl or cycloalkylalkenyl preferably is of 3 to 7 carbon atoms in the cycloalkyl, particularly 5 or 6 carbon atoms, and of 1 to 5 carbon atoms, particularly 1 carbon atom, in the alkyl or alkylene moieties thereof. Aryl preferably is phenyl or naphthyl, especially 2-naphthyl. Heteroaryl preferably is pyridinyl, thienyl, especially 2-thienyl, or furyl, especially 2-furyl. Heteroarylalkyl and heteroalkenyl preferably has 1 to 6 carbon atoms, especially 1 carbon atom in the alkyl or alkylene moieties thereof. The heteroaryl moiety of heteroarylalkyl and heteroarylalkylene preferably has the significances indicated above as preferred for heteroaryl. Aralkyl preferably is phenylalkyl of 7 to 12 carbon atoms, particularly naphthylmethyl, benzyl and phenylethyl. Alkoxy preferably is of 1 to 5 carbon atoms, preferably methoxy. Acyloxy preferably is of 2 to 6 carbon atoms, preferably acetoxy. The optional substituents of an aryl or aralkyl moiety preferably are one to three radicals of alkyl of 1 to 5 carbon atoms, alkoxy of 1 to 5 carbon atoms, halogen of atomic number of from 9 to 35, hydroxy and/or amino, preferably one or two groups methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, particularly one hydroxy, amino, chlorine, bromine, or fluorine, optionally in protected form where appropriate, halogen-substituted alkyl of 1 to 5 carbon atoms, particularly trifluoromethyl.

Specifically excluded from the definition of amino acid side chain is oxo. Thus, the ethylenediamine does not contain peptide bonds.

The peptidyl functionality may also be depicted as shown in Formulae (a) and (b):



in which n is 0 to 9; X_3X_4 represents a linkage selected from $-C(O)NR_{14}-$, $-CH_2NR_{14}-$, $-C(O)CH_2-$ and $-NR_{14}C(O)-$; Y is $-CH(R_{14})-$ or $-NR_{14}-$; and Z is $-(CH_2)_2-$, $-C(R_{15})(R_{16})-$ or $-N(R_{16})-$; wherein R_{14} is hydrogen or as defined below, R_{15} is hydrogen or methyl and each R_{16} is an amino acid side chain. Thus each R_{16} is independently hydrogen, alkyl (optionally substituted with a radical selected from hydroxy, alkyloxy, amino, alkylamino, dialkylamino, uriedo, alkyluriedo, mercapto, alkylthio, carboxy, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkylsulfinyl, alkylsulfonyl, guanidino, $-P(O)(OR_{12})_2$, $-OP(O)(OR_{12})_2$ or $-OP(O)(R_{12})_2$, wherein each R_{12} is independently hydrogen or alkyl, or 10 a protected derivative thereof), cycloalkyl, cycloalkylalkyl, a group selected from aryl and arylalkyl (which group is optionally substituted at its aryl ring with one to three radicals selected from hydroxy, amino, guanidino, halo, optionally halo-substituted alkyl, alkyloxy and aryl, or a protected derivative thereof) or together with an adjacent R_{14} forms a divalent radical selected from (C_{3-4}) methylene and 15 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo).

The peptidyl group may also contain additional functional groups, as depicted by "PG" of Formula 3 below. Thus, the protecting group, "PG", of Formula 3 may be a peptide amino end blocking group or a label, as these terms are defined below. By the term "peptide amino end blocking group" herein is meant, for example, groups including, but not limited to alkyloxycarbonylalkanoyl (preferably of overall 2 to 10 carbon atoms), alkyloxycarbonyl (preferably of overall 2 to 10 carbon atoms and particularly *tert*-butoxycarbonyl (BOC) or benzyloxycarbonyl (CBZ, Z)), alkanoyl (preferably of overall 2 to 10 carbon atoms and optionally substituted with a radical selected from carboxy, 25 alkyloxycarbonyl and heterocycloalkylalkanoylamino), cycloalkylcarbonyl (preferably of overall 4 to 8 carbon atoms), heterocycloalkylcarbonyl (preferably of overall 6 to 10 atoms and optionally substituted with a radical selected from hydroxy, alkyl, heterocycloalkyl, alkanoyl, alkyloxycarbonyl, arylalkyloxycarbonyl and

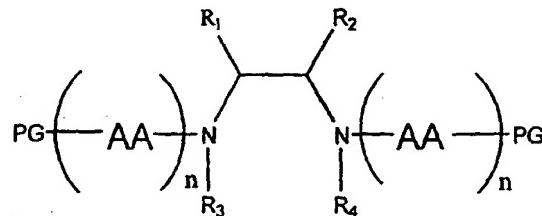
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heterocycloalkylcarbonyl and particularly a heterocycloalkylcarbonyl group of the formula -C(O)NR₁₀R₁₁, wherein R₁₀ and R₁₁, together form aza(C₂₋₆)methylene, oxa(C₂₋₆)methylene or (C₃₋₇)methylene, particularly oxapentamethylene to form 4-morpholinylcarbonyl (Mu), arylalkyloxycarbonyl (preferably comprising aryl of 6 to 10 carbon atoms and alkyloxy of 1 to 5 carbon atoms), carbamoyl, alkylcarbamoyl (preferably of overall 2 to 6 carbon atoms), dialkylcarbamoyl (preferably of overall 2 to 11 carbon atoms, arylcarbamoyl (preferably of overall 7 to 11 carbon atoms), arylalkylcarbamoyl (preferably comprising aryl of 6 to 10 carbon atoms and alkyl of 1 to 5 carbon atoms), arylalkanoyl (preferably comprising aryl of 6 to 10 carbon atoms and alkanoyl of overall 1 to 6 carbon atoms), aroyl (preferably of overall 7 to 11 carbon atoms and particularly benzoyl), alkylsulfonyl (preferably of 1 to 10 carbon atoms), arylalkylsulfonyl (preferably comprising aryl of 6 to 10 carbon atoms and alkyl of 1 to 5 carbon atoms), alkylsulfamoyl (preferably of 1 to 5 carbon atoms), dialkylsulfamoyl (preferably of 2 to 10 carbon atoms), arylsulfonyl (of 6 to 10 carbon atoms, including heteroarylsulfonyl, preferably comprising heteroaryl of overall 4 to 8 atoms), arylsulfamoyl (preferably of 6 to 10 carbon atoms, including heteroarylsulfamoyl preferably comprising heteroaryl of 4 to 8 atoms), alkylsulfinyl (preferably of 1 to 5 carbon atoms), dialkylaminosulfinyl (preferably of 2 to 10 carbon atoms), arylsulfinyl (preferably comprising aryl of 6 to 10 carbon atoms including heteroarylsulfinyl, preferably comprising heteroaryl of 4 to 8 atoms). Temporary protecting groups are known in the art, see Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, 1991, hereby incorporated by reference.

Formula 3 depicts a cysteine protease inhibitor with peptidyl groups in both the A and X positions, wherein n is from 1 to 5:

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Formula 3

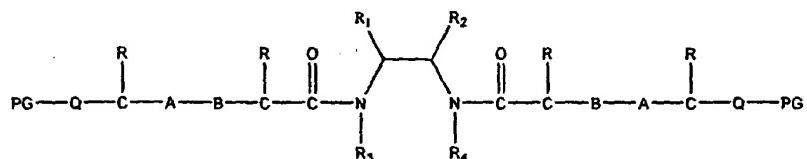


The amino acids, or peptide residues, are normally linked via a peptide bond or linkage, i.e. a peptidic carbamoyl group, i.e. -CONH-. In a preferred embodiment, the

bond between the nitrogens of the ethylenediamine or piperazine moiety and the A and X group is a peptide bond. Similarly, when the peptidyl group contains two or more amino acids, the bond between the two is preferably a peptide bond. However, the bond between the amino acids of the peptidyl group may be a peptide isosteric or peptidomimetic bond such as CO-CH₂, CH₂-NH, azapeptide and retro-inverso bonds, as is depicted by X₃-X₄ in Formulae (a) and (b).

5

Formula 4



Formula 4 depicts a cysteine protease inhibitor with peptidyl groups comprising two amino acids as both the A and the X group. Thus, in formula 4, the A-B linkage is a peptide or peptidomimetic bond. PG is a protecting group, as defined herein, and Q may be either carbon or nitrogen. As will be appreciated, additional amino acids may be added in the same manner. Similarly, it should be understood that these structures do not accurately reflect the situation where one or more of the amino acids is a proline.

10

15 In general, suitable amino acids of the peptidyl groups of the invention are initially chosen on the basis of the rules governing substrate specificity in cysteine proteases. That is, combinations of amino acids that confer specificity to the enzyme to be inhibited will be used.

20 It is to be understood that the order of the amino acid side chains within the inhibitor is significant in conferring inhibitor targeting. Thus, as is additionally described below, the amino acid side chain attached to the ethylenediamine or piperazine structure of the inhibitor, generally referred to herein as "R," or "R₂," will occupy the S₁ position of the enzyme's substrate binding site when the inhibitor is bound to the enzyme. That is, for example, the "R₂" amino acid side chain of the targeting group is the P₁ residue of the inhibitor. Similarly, if there is a peptidyl group in the A position, the amino acid side chain of the peptidyl group which is closest to the ethylenediamine moiety will occupy the S₂ position of the enzyme's substrate binding site when the inhibitor is bound to the

25

enzyme, and thus is the P₂ residue. If present, additional amino acid side chains of the peptidyl will occupy the P₃, P₄, etc. positions.

Conversely, if the peptidyl group occupies the X position, the amino acid side chains occupy the S₁, S₂, etc. position of the enzyme's substrate binding site. These amino acid side chains are considered the P₁, P₂, etc. residues of the inhibitor. As will be appreciated by those in the art, it is possible to have peptidyl groups at both the A and X positions, to confer increased specificity on the inhibitor for the particular cysteine protease to be inhibited.

It should be understood that the inhibitors of the present invention potentially have a certain symmetry which could effect nomenclature. Thus, for example, if the A and the X groups are identical, the inhibitor can be thought of as having an R₁ group in one conformation or an R₂ group in the opposite conformation. Without being bound by theory, the A group is assumed to be binding in the P positions, and the X group to be binding to the P' positions of the enzyme.

The choice of the amino acid side chains of the R₁, R₂, R₅, R₆ and R₁₆ group, and the amino acids of the A and X groups will be made using the available information about the substrate specificity of the protease, and is routine to those skilled in the art using commercially available substrates. For example, interleukin-1 β converting enzyme displays the greatest specificity demonstrated for a cysteine protease toward a substrate, requiring an aspartyl side chain in the P₁ position. The papain superfamily of cysteine proteases have an extended specificity site containing five to seven significant subsites, with the dominant one being S₂, which is a hydrophobic pocket that binds phenylalanyl-like sidechains very well. Cathepsin B, similar to papain, accepts a phenylalanine side chain in S₂, as well as an arginyl sidechain. For a general review, see "Proteinase Inhibitors", in Research Monographs in Cell and Tissue Physiology (1986), ed. Barrett et al., Vol. 12, Chapter 4: Inhibitors of Cysteine Proteinases, Daniel Rich, Elsevier, New York, hereby expressly incorporated by reference. In addition, the specificity of the interleukin 1 β converting enzyme (ICE), was explored in Thornberry et al., supra, also expressly incorporated by reference herein. Table 1 lists some of the favored amino acid side chains for the P₁ and P₂ (R₁ or R₂) positions for a number of cysteine proteases.

Table 1

enzyme	P ₂	P ₁ , (R ₁ or R ₂)
papain	Phe, Tyr, 2-naphthyl, Leu, Nle, Ile, Ala	Arg, Lys, Lys(e-Z), guanidino-phenylalanine, Hph, Nle
cathepsin B	Phe, Tyr, Tyr(I ₂), 2-naphthyl, Arg, guanidino-phenylalanine, Cit*	Arg, Lys, Lys(e-Z), guanidino-phenylalanine, Hph, Cit, Nle
5 cathepsin L or cruzain	Phe, Tyr, 2-naphthyl	Arg, Lys, Lys(e-Z), guanidino-phenylalanine, Hph, Cit, Nle
cathepsin S	Phe, Tyr, 2-naphthyl, Val, Leu, Nle, Ile, Ala	Arg, Lys, Lys(e-Z), guanidino-phenylalanine, Hph, Cit, Nle
DPP-1	Gly, Ala	Phe, Tyr
calpain	Val, Leu, Nle, Ile, Phe	Tyr, Phe, Met, Met(O ₂), Val
10 ICE	Ala, Val, His	Asp
cathepsin O2	Leu, Met, Nle	Arg, Lys, Lys(e-Z), guanidino-phenylalanine, Hph, Nle

*citrulline

As will be appreciated by those in the art, when non-naturally occurring amino acid side chain analogs are used, they will be chosen initially by steric and biochemical similarities to the naturally occurring side chains.

By "alkyloxycarbonyl" herein is meant a -COOR₆ group, wherein C is carbon and O is oxygen. In this embodiment, suitable R₆ groups include, but are not limited to, an alkyl, a cycloalkyl, a cycloalkylalkyl, an aryl, or an aralkyl (forming an arylalkyloxycarbonyl). In such an instance, alkyl is preferably of 1 to 5 carbon atoms, especially ethyl. Cycloalkyl preferably is of 3 to 7 carbon atoms, preferably cyclopentyl or cyclohexyl. Cycloalkylalkyl preferably is of 3 to 7 carbon atoms in the cycloalkyl, particularly 5 or 6 carbon atoms, and of 1 to 5 carbon atoms, particularly 1 carbon atom, in the alkyl moieties thereof. Aryl preferably is phenyl. Aralkyl preferably is phenylalkyl of 7 to 12 carbon atoms, particularly benzyl. The optional substituents of an aryl or aralkyl moiety preferably are one or two groups independently selected from: alkyl of 1 to 5 carbon atoms, alkoxy of 1 to 5 carbon atoms, halogen of atomic number of from 9 to 35,

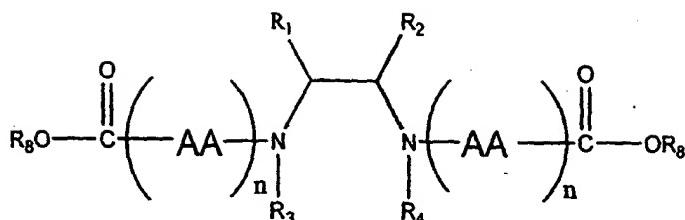
hydroxy and/or amino, preferably one or two groups methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, particularly one hydroxy, amino, chlorine, bromine, or fluorine, optionally in protected form where appropriate, nitro, alkyl or arylsulfonyl, or halogen-substituted alkyl of 1 to 5 carbon atoms, particularly
5 trifluoromethyl.

Particularly preferred are: (1) C1-C5 alkyl, especially ethyl; (2) C3-C7 cycloalkyl, preferably cyclopentyl or cyclohexyl; (3) C3-C7(cycloalkyl) - C1-C5 alkyl, preferably C5-C6(cycloalkyl)methyl; (4) C3-C7(cycloalkyl-alkenyl)-C1-C5 alkyl, preferably C5-C6(cycloalkylalkenyl)methyl; (5) phenyl; (6) C7-C12 phenylalkyl, preferably benzyl; (7)
10 C1-C5 alkyl substituted by C1-C5 alkoxy, halogen, hydroxy or amino, with C1-C5 alkyl preferably substituted by one or two groups selected from methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, with hydroxy, amino, chlorine, bromine or fluorine being most preferred; (8) C1-C5 alkyl substituted with nitro, alkyl or arylsulfonyl, optionally protected where appropriate; (9) C1-C5 alkyl substituted with
15 halogen, preferably trifluoromethyl; (10) aryl or aralkyl substituted by one or two groups of C1-C5 alkyl, C1-C5 alkoxy, halogen, hydroxy or amino, with one or two groups of methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino being preferred and hydroxy, amino, chlorine, bromine or fluorine being particularly preferred; (11) aryl or aralkyl substituted by one or two groups of halogen-substituted C1-C5 alkyl, especially
20 trifluoromethyl; nitro; sulfonyl; or arylsulfonyl, in protected form where appropriate.

Preferred alkyloxycarbonyls include, but are not limited to, Boc, CBZ and Z.

By "peptidyl alkyloxycarbonyl" or "alkyloxycarbonyl peptidyl" herein is meant a peptidyl group linked to a alkyloxycarbonyl group. It is to be understood that the peptidyl group is linked to one of the nitrogens of the ethylenediamine or piperazine moieties, and the
25 alkyloxycarbonyl group is linked to the peptidyl group. Thus, the free terminal functionality of a alkyloxycarbonyl peptidyl group is the R₈ group of Formula 5. As described above for acyl peptidyl, the order of the functionalities differs depending on whether it is the A group or the X group which is the alkyloxycarbonyl peptidyl; the group may be called a alkyloxycarbonyl peptidyl moiety when describing the A group
30 and a peptidyl alkyloxycarbonyl moiety when describing the X group. Formula 5 shows a cysteine protease inhibitor with alkyloxycarbonyl peptidyl groups at both the A and X positions, wherein n is from 1 to 5, and AA is an amino acid:

Formula 5



By "sulfonyl" herein is meant an $-SO_2R_9$ group, wherein S is sulfur, and O is oxygen.

The sulfur atom is attached to one of the nitrogens of the ethylenediamine or piperazine moieties, and thus the sulfonyl group is also a sulfonamide because of the

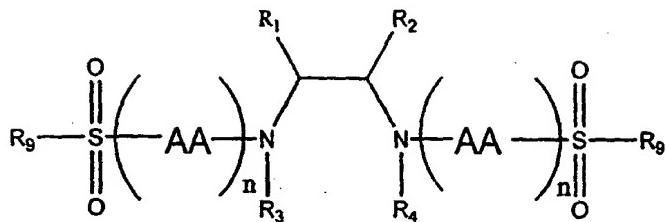
- 5 attachment to the nitrogen group. The R₉ moiety of the sulfonyl group may include, but is not limited to, an alkyl (forming alkylsulfonyl), a substituted alkyl, a cycloalkyl, a cycloalkylalkyl, a cycloalkylalkenyl, an aryl (forming arylsulfonyl, including heteroaryl (forming heteroarylsulfonyl)), or an aralkyl (forming arylalkylsulfonyl). In such an instance, alkyl is preferably of 1 to 5 carbon atoms, especially methyl. Substituted alkyl is preferably of 1 to 5 carbon atoms, bearing substitutions of alkoxy of 1 to 5 carbon atoms, halogen of atomic number of from 9 to 35, hydroxy and/or amino, preferably one or two groups methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, particularly one hydroxy, amino, chlorine, bromine, or fluorine, optionally in protected form where appropriate, nitro, alkyl or arylsulfonyl, or halogen-substituted alkyl of 1 to 5 carbon atoms, particularly trifluoromethyl. Cycloalkyl preferably is of 3 to 7 carbon atoms, preferably cyclopentyl or cyclohexyl. Cycloalkylalkyl or cycloalkylalkenyl preferably is of 3 to 7 carbon atoms in the cycloalkyl, particularly 5 or 6 carbon atoms, and of 1 to 5 carbon atoms, particularly 1 carbon atom, in the alkyl or alkylene moieties thereof. Aryl preferably is phenyl, pentafluorophenyl or naphthyl.
- 10 Aralkyl preferably is phenylalkyl of 7 to 12 carbon atoms, particularly benzyl and phenethyl. The optional substituents of an aryl or aralkyl moiety preferably are one or two groups alkyl of 1 to 5 carbon atoms, alkoxy of 1 to 5 carbon atoms, halogen of atomic number of from 9 to 35, hydroxy and/or amino, preferably one or two groups methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, particularly one
- 15 hydroxy, amino, chlorine, bromine, or fluorine, optionally in protected form where appropriate, nitro, alkyl or arylsulfonyl, or halogen-substituted alkyl of 1 to 5 carbon atoms, particularly trifluoromethyl.
- 20
- 25

Particularly preferred R₉ moieties of the sulfonyl group include (1) C1-C5 alkyl, especially methyl; (2) C3-C7 cycloalkyl, preferably cyclopentyl or cyclohexyl; (3) C3-C7(cycloalkyl)-C1-C5 alkyl, preferably C5-C6(cycloalkyl)methyl; (4) C3-C7(cycloalkylalkenyl)-C1-C5 alkyl, preferably C5-C6(cycloalkylalkenyl)-methyl; (5) phenyl, preferably pentafluorophenyl or naphthyl; (6) C7-C12 phenylalkyl, preferably benzyl; (7) C1-C5 alkyl substituted by C1-C5 alkoxy, halogen, hydroxy or amino, with C1-C5 alkyl preferably substituted by one or two groups selected from methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, with hydroxy, amino, chlorine, bromine or fluorine being most preferred; (8) C1-C5 alkyl substituted with nitro, alkyl or arylsulfonyl, optionally protected where appropriate; (9) C1-C5 alkyl substituted with halogen, preferably trifluoromethyl; (10) aryl or aralkyl substituted by one or two groups of C1-C5 alkyl, C1-C5 alkoxy, halogen, hydroxy or amino, with one or two groups of methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino being preferred and hydroxy, amino, chlorine, bromine or fluorine being particularly preferred; (11) aryl or aralkyl substituted by one or two groups of halogen-substituted C1-C5 alkyl, especially trifluoromethyl; nitro; sulfonyl; or arylsulfonyl, in protected form where appropriate.

Especially preferred are phenyl, naphthyl, and benzyl.

By "peptidyl sulfonyl" or "sulfonyl peptidyl" herein is meant a peptidyl group linked to a sulfonyl group. As above, the peptidyl group is linked to one of the nitrogens of the ethylenediamine or piperazine moieties, and the sulfonyl group is linked to the peptidyl group. Thus, the free terminal functionality of a sulfonyl peptidyl group is the R₉ group. As described above for acyl peptidyl, the order of the functionalities differs depending on whether it is the A group or the X group which is the sulfonyl peptidyl; the group may be called a sulfonyl peptidyl moiety when describing the A group and a peptidyl sulfonyl moiety when describing the X group. Formula 6 shows a cysteine protease inhibitor with sulfonyl peptidyl groups at both the A and X positions, wherein n is from 1 to 5, and AA is an amino acid.

Formula 6



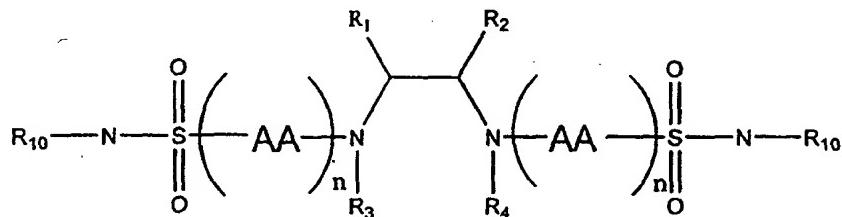
By "sulfamoyl" herein is meant an $-\text{SO}_2\text{NR}_{10}\text{R}_{11}$ group, wherein S is sulfur, O is oxygen, and N is nitrogen. The sulfur atom of the sulfone is linked to one of the nitrogens of the ethylenediamine or piperazine moieties. In some embodiments, the sulfamoyl 5 comprises an $-\text{SO}_2\text{NHR}_{10}$ group, wherein H is hydrogen, and in other embodiments it comprises an $-\text{SO}_2\text{NR}_{10}\text{R}_{11}$ group.

Suitable $-\text{NHR}_{10}$ and $-\text{NR}_{10}\text{R}_{11}$ groups include, but are not limited to, an NH_2 , or an NH-alkyl (forming alkylsulfamoyl), an NH-cycloalkyl, an NH-cycloalkylalkyl, an NH-aryl (forming arylsulfamoyl or heteroaryl sulfamoyl), an NH-aralkyl, N-dialkyl (forming dialkylsulfamoyl) and N-alkylaralkyl. Cycloalkyl preferably is of 3 to 7 carbon atoms, preferably cyclopentyl or cyclohexyl. Cycloalkylalkyl preferably is of 3 to 7 carbon atoms in the cycloalkyl, particularly 5 or 6 carbon atoms, and of 1 to 5 carbon atoms, particularly 1 carbon atom, in the alkyl moieties thereof. Aryl preferably is phenyl or heteroaryl. Aralkyl preferably is phenylalkyl of 7 to 12 carbon atoms, particularly benzyl. The optional substituents of an aryl or aralkyl moiety preferably are one or two groups alkyl of 1 to 5 carbon atoms, alkoxy of 1 to 5 carbon atoms, halogen of atomic number of from 9 to 35, hydroxy and/or amino, preferably one or two groups methyl, methoxy, chlorine, bromine, fluorine, hydroxy or amino, particularly one hydroxy, amino, chlorine, bromine, or fluorine, optionally in protected form where appropriate, 10 nitro, alkyl or arylsulfonyl, or halogen-substituted alkyl of 1 to 5 carbon atoms, particularly trifluoromethyl. Especially preferred is when both R_{10} and R_{11} are methyl.

In a preferred embodiment, the R_{10} and R_{11} groups of a $-\text{NR}_{10}\text{R}_{11}$ are bonded together to form 5 or 6 membered alicyclic or heterocyclic ring moieties. Preferred are piperidine, morpholine, pyrrolidine, piperazine, or substituted piperazine.

By "peptidyl sulfamoyl" herein is meant a peptidyl group linked to a sulfamoyl group. As above, the peptidyl group is linked to one of the nitrogens of the ethylenediamine or piperazine moieties, and the sulfamoyl group is linked to the peptidyl group. Thus, the free terminal functionality of a sulfamoyl peptidyl group is the R₁₀ group. As described 5 above for acyl peptidyl, the order of the functionalities differs depending on whether it is the A group or the X group which is the sulfamoyl peptidyl; the group may be called a sulfamoyl peptidyl moiety when describing the A group and a peptidyl sulfamoyl moiety when describing the X group. Formula 7 shows a cysteine protease inhibitor with a sulfamoyl peptidyl group at the A position and a peptidyl sulfamoyl at the X 10 position, wherein n is from 1 to 10, and AA is an amino acid.

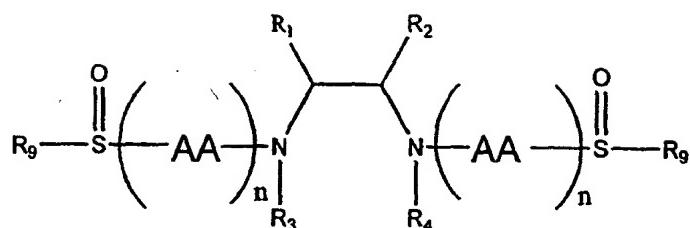
Formula 7



By "sulfinyl" herein is meant a -SOR₉ group, where S is sulfur, O is oxygen, and R₉ is a group as defined herein. The sulfur and oxygen atoms are double bonded together. The sulfur atom is attached to one of the nitrogens of the ethylenediamine or 15 piperazine moieties. Preferred sulfinyl groups include alkylsulfinyl, dialkylaminosulfinyl, and arylsulfinyl, including heteroarylsulfinyl.

By "sulfinyl peptidyl" herein is meant a peptidyl group linked to a sulfinyl group. As above, the peptidyl group is linked to one of the nitrogens of the ethylenediamine or piperazine moieties, and the sulfinyl group is linked to the peptidyl group. Thus, the 20 free terminal functionality of a sulfinyl peptidyl group is the R₉ moiety. As described above, the order of the functionalities differs depending on whether it is the A group or the X group which is the sulfinyl peptidyl; the group may be called a sulfinyl peptidyl group when describing the A moiety, and a peptidyl sulfinyl when describing the X group. Formula 8 depicts a cysteine protease inhibitor with a sulfinyl peptidyl group at the A position and a peptidyl sulfinyl at the X position, wherein n is from 1 to 10, and 25 AA is an amino acid.

Formula 8

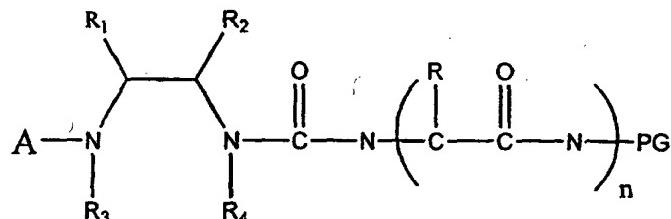


By "carbamoyl" herein is meant an -CONR₁₀R₁₁ group, H is hydrogen, C is carbon and O is oxygen. R₁₀ and R₁₁ are defined as above.

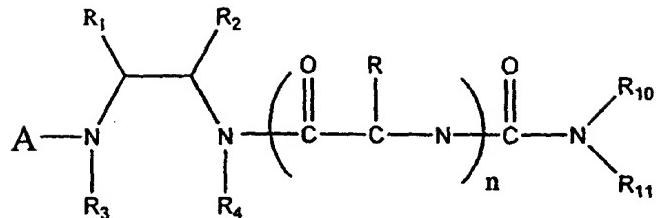
- By "carbamoyl peptidyl" herein is meant a carbamoyl group linked to a peptidyl group.
- 5 The term "carbamoyl peptidyl" includes both "carbamoyl peptidyl" and "peptidyl carbamoyl". That is, in one embodiment the carbamoyl is attached to one of the nitrogens of the ethylenediamine or piperazine moieties, and the peptidyl moiety is attached to the carbamoyl. In this embodiment, the terminal functionality is the peptidyl. Alternatively, the peptidyl moiety may be attached to the ethylenediamine or
- 10 piperazine moiety and the carbamoyl moiety attached to the peptidyl. In this embodiment, the terminal functionality is the carbamoyl. These two embodiments are depicted below in Formulas 9 (carbamoyl peptidyl) and 10 (peptidyl carbamoyl) as comprising the X moiety. It should be understood that Formulas 9 and 10 do not accurately reflect the structure where one of the amino acids is a proline.

15

Formula 9

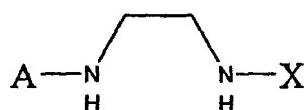


Formula 10



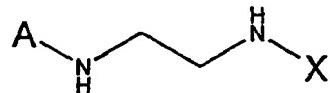
The two N-substituents of the invention are linked via an ethylenediamine or substituted ethylenediamine group. By "ethylenediamine group" herein is meant a -NH-CH₂-CH₂-NH- group, wherein N is nitrogen, C is carbon, and H is hydrogen. The two N-substituents, depicted herein as "A" and "X", are each linked to one of the nitrogens of the ethylenediamine. "Linked" herein means a covalent attachment. Thus, in one embodiment, the cysteine protease inhibitors of the invention have the structure shown in Formula 11:

10 Formula 11



It should be understood that the depiction of the ethylenediamine group in Formula 11 and others as having a certain conformation is merely pictorial. Thus, Formula 11, and the other ethylenediamine moieties may be depicted as shown in Formula 11A:

Formula 11A

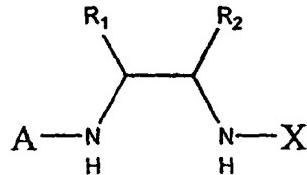


- 15 In a preferred embodiment, the N-substituents are linked via a substituted ethylenediamine moiety. By "substituted ethylenediamine" herein is meant an ethylenediamine group which has one or more of the hydrogen atoms replaced by a substituent group. It should be understood that the substituted ethylenediamine does not contain a peptide bond; that is, neither the R₁ or the R₂ positions may be a carbonyl.
- 20

In a preferred embodiment, the ethylenediamine is substituted with an R group on either one of the carbons of the ethylene. Thus, as shown in Formula 12, R₁ and R₂ are either hydrogen or an amino acid side chain. It is to be understood that only one of R₁ and R₂ is an amino acid side, and the other one of R₁ and R₂ is hydrogen.

- 5 Preferably, R₂ is an amino acid side chain and R₁ is hydrogen.

Formula 12



As will be appreciated by those in the art, there are a large number of possible A and X combinations which can be made. Any A group may be combined with any X group.

- 10 In one embodiment, the A and the X group are the same; for example, the A group may be a sulfonyl peptidyl group and the X group may be a peptidyl sulfonyl group. However, it should be understood that even if both the A and X groups are sulfonyls, for example, the R₉ groups of each sulfonyl need not be the same. This is similarly true for the other embodiments. In an alternative embodiment, the A group and the X group are different.

- 15 When R₂ is an amino acid side chain and R₁ is hydrogen, preferred A and X group combinations are depicted in Table 2:

Table 2

	Preferred A group	Preferred X group
	PAEBG-amino acid*	sulfonyl
20	PAEBG-peptidyl	sulfonyl
	alkyloxycarbonyl peptidyl	sulfonyl
	sulfonyl peptidyl	sulfonyl
	carbamoyl peptidyl	sulfonyl
	sulfamoyl peptidyl	sulfonyl
25	acyl peptidyl	sulfonyl
	sulfinyl peptidyl	sulfonyl

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PG-amino acid	peptidyl-PAEBG
alkyloxycarbonyl peptidyl	peptidyl-PAEBG
sulfonyl peptidyl	peptidyl-PAEBG
carbamoyl peptidyl	peptidyl-PAEBG
5 sulfamoyl peptidyl	peptidyl-PAEBG
acyl peptidyl	peptidyl-PAEBG
sulfinyl peptidyl	peptidyl-PAEBG

*PAEBG = peptide amino end blocking group, or PG

When R₁ is an amino acid side chain and R₂ is hydrogen, preferred A and X group combinations are depicted in Table 3:

Table 3

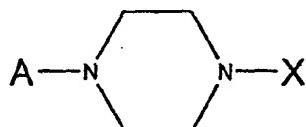
Preferred A group	Preferred X group
sulfonyl	peptidyl-PAEBG
15 sulfonyl	peptidyl alkyloxycarbonyl
sulfonyl	peptidyl sulfonyl
sulfonyl	peptidyl carbamoyl
sulfonyl	peptidyl sulfamoyl
sulfonyl	peptidyl acyl
sulfonyl	peptidyl sulfinyl
20 PAEBG peptidyl	amino acid-PG
PAEBG peptidyl	peptidyl alkyloxycarbonyl
PAEBG peptidyl	peptidyl sulfonyl
PAEBG peptidyl	peptidyl carbamoyl
PAEBG peptidyl	peptidyl sulfamoyl
25 PAEBG peptidyl	peptidyl acyl
PAEBG peptidyl	peptidyl sulfinyl

The preferred A and X groups listed in Tables 2 and 3 apply to the piperazine moieties described below as well.

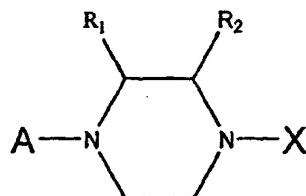
In a preferred embodiment, the ethylenediamine is substituted on the nitrogens of the ethylenediamine, as shown in Formula 1. In this embodiment, R₃ and R₄ are either both hydrogen or are bonded together to form ethylene or substituted ethylene. By "ethylene" or "ethylene group" herein is meant a (-CH₂CH₂-) group, i.e. a saturated carbon-carbon bond, serving to connect two sp³-hybridized carbon atoms; that is, the two carbons of the ethylene group are not double bonded together. By "substituted ethylene" herein is meant an ethylene group which has one of the hydrogens of the ethylene replaced by a substituent group, i.e. a (-CH₂CHR-) group, where R is R₅ or R₆, as depicted below. When R₃ and R₄ form ethylene or substituted ethylene, the substituted ethylenediamine moiety is a piperazine moiety, which in turn may be substituted or unsubstituted. That is, the carbon atoms of the piperazine moiety may have no substituent groups, i.e. only hydrogen, as shown in Formula 13, an R₁ or R₂ group as shown in Formula 14, an R₅ or R₆ group as shown in Formula 15, or both an R₁ and R₂ or an R₂ and R₅ group (Formulas 16 and 17).

15

Formula 13

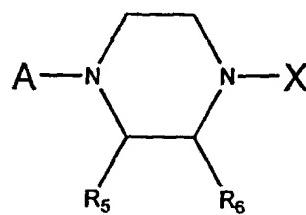


Formula 14



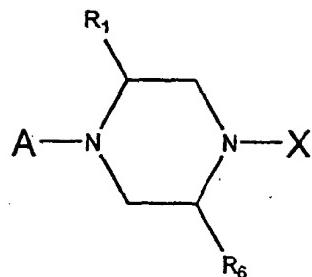
In a preferred embodiment, R₂ is an amino acid side chain and R₁ is hydrogen.

Formula 15

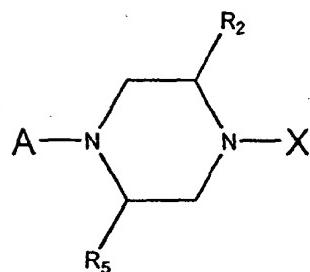


-33-

Formula 16



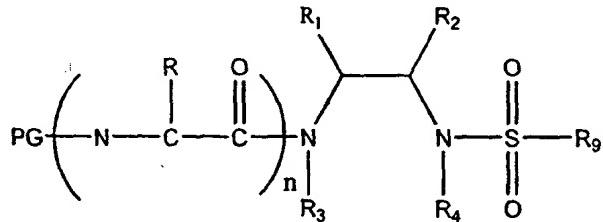
Formula 17



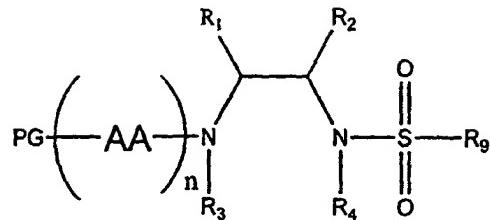
- In Formulas 16 and 17, it is to be understood that the two substitution groups of the six-membered heterocyclic ring of piperazine are in para position to each other; that is, there may be substitution groups at R₁ and R₆ or R₂ and R₅. R₁, R₂, R₅ and R₆ are independently hydrogen or an amino acid side chain.

- In a preferred embodiment, the cysteine protease inhibitors of the present invention have a peptidyl moiety as the "A" group and a sulfonyl moiety as the "X" group, and an R₂ group, and thus have the general formula shown in Formula 18 and Formula 18A.
- As will be appreciated by those in the art, Formula 18 does not accurately represent the structure when one or more of the amino acids of the peptidyl group are proline.

Formula 18



Formula 18A

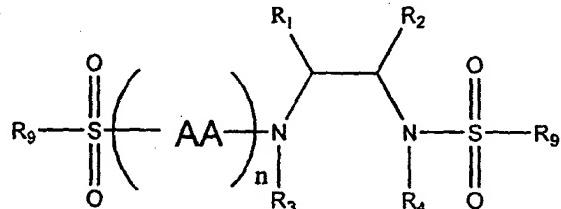


When the cysteine protease inhibitors have the formula depicted in Formulas 18 and 18A, preferably the inhibitors have an R₂ group. In addition, preferably n = 1 or 2. In one preferred embodiment, R₃ and R₄ are hydrogen; in an alternate preferred

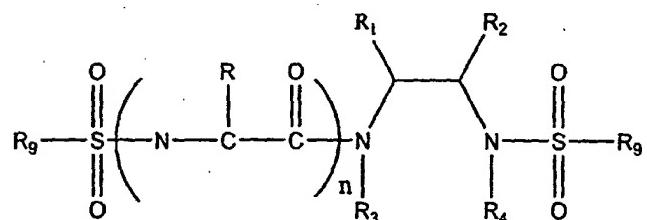
5 embodiment, R₃ and R₄ together form unsubstituted ethylene, to form a piperazine which has a single substituent group at R₂.

In a preferred embodiment, the cysteine protease inhibitors have a sulfonyl peptidyl moiety as the "A" group and a sulfonyl moiety as the "X" group, and an R₂ group, and thus have the general formula shown in Formula 19 and 19A (where the amino acid is
10 not proline):

Formula 19



Formula 19A



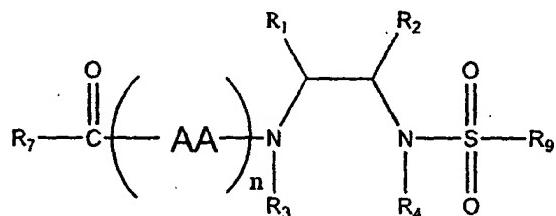
When the cysteine protease inhibitors have the formula depicted in Formula 19 and 19A, preferably the inhibitors have an R₂ group. In addition, preferably n = 1 or 2. As above, this structure is not accurate when one or more of the amino acids of the peptidyl are proline. In one embodiment, R₃ and R₄ are hydrogen; in a preferred embodiment, R₃ and R₄ together form unsubstituted ethylene, to form a piperazine which has a single substituent group at R₂.

5

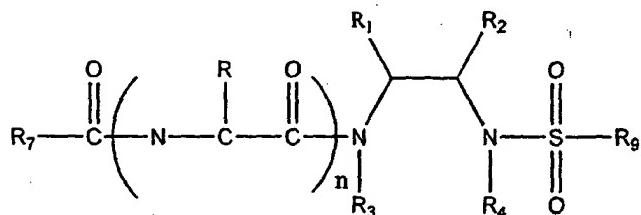
In a preferred embodiment, the cysteine protease inhibitors of the invention have an acyl peptidyl moiety as the "A" group and a sulfonyl moiety as the "X" group as depicted as Formula 20 and Formula 20A (when the amino acid is not proline):

10

Formula 20



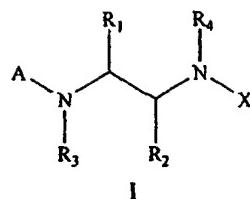
Formula 20A



When the cysteine protease inhibitors have the formula depicted in Formula 20 and 20A, preferably the inhibitors have a R₂ group. Preferably n=1 or 2. In one embodiment, R₃ and R₄ are hydrogen; in an alternative embodiment R₃ and R₄ together form unsubstituted ethylene, to form a piperazine which has a single substituent group at R₂.

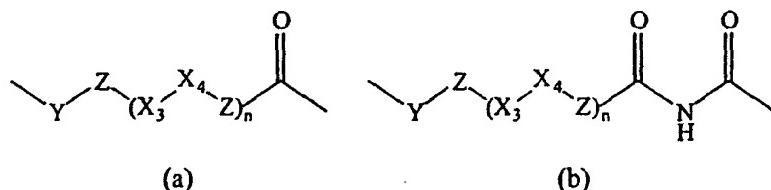
15

Thus, preferred embodiments include compounds of Formula I:



in which:

A and X are independently R₁₃-X₁, wherein R₁₃ is hydrogen, alkyloxycarbonylalkanoyl, alkyloxycarbonyl, alkanoyl (optionally substituted with a radical selected from carboxy, 5 alkyloxycarbonyl and heterocycloalkylalkanoylamino), cycloalkylcarbonyl, heterocycloalkylcarbonyl (optionally substituted with a radical selected from hydroxy, alkyl, heterocycloalkyl, alkanoyl, alkyloxycarbonyl, arylalkyloxycarbonyl and heterocycloalkylcarbonyl), arylalkyloxycarbonyl, carbamoyl, alkylcarbamoyl, 10 dialkylcarbamoyl, arylcarbamoyl, arylalkylcarbamoyl, arylalkanoyl, aroyl, alkylsulfonyl, arylsulfonyl, arylalkylsulfonyl, alkylsulfamoyl, dialkylsulfamoyl, arylsulfamoyl, heteroarylsulfamoyl, alkylsulfinyl, dialkylaminosulfinyl or arylsulfinyl and X₁ is a bond or a divalent radical of Formulae (a) or (b):



15 in which n is 0 to 9; X_3 - X_4 represents a linkage selected from -C(O)NR₁₄-, -CH₂NR₁₄-,
-C(O)CH₂- and -NR₁₄C(O)-; Y is -CH(R₁₄)- or -NR₁₄-; and Z is -(CH₂)₂- , -C(R₁₅)(R₁₆)- or
-N(R₁₆); wherein R₁₄ is hydrogen or as defined below, R₁₅ is hydrogen or methyl and
each R₁₆ is independently hydrogen, alkyl (optionally substituted with a radical selected
from hydroxy, alkyloxy, amino, alkylamino, dialkylamino, ureido, alkylureido, mercapto,
alkylthio, carboxy, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkylsulfinyl,
20 alkylsulfonyl, guanidino, -P(O)(OR₁₂)₂, -OP(O)(OR₁₂)₂ and -OP(O)(R₁₂)₂, wherein each
R₁₂ is independently hydrogen or alkyl, or a protected derivative thereof), cycloalkyl,
cycloalkylalkyl, a group selected from aryl and arylalkyl (which group is optionally

substituted at its aryl ring with one to three radicals selected from hydroxy, amino, guanidino, halo, optionally halo-substituted alkyl, alkyloxy and aryl, or a protected derivative thereof) or together with an adjacent R₁₄ forms a divalent radical selected from (C₃₋₄)methylene and 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo), with the proviso X and A are not both hydrogen;

R₁ and R₂ are both hydrogen or one of R₁ or R₂ is cyano, carboxy, alkyloxycarbonyl, alkanoyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkyloxy(alkyl)carbamoyl, aminoalkylcarbamoyl, R₁₆, as defined above, or R₁₃-X₂₋, wherein R₁₃ is as defined above and X₂ is a divalent radical of Formulae (a) or (b), as defined above; and R₃ and R₄ together form optionally substituted ethylene or are independently R₁₄, as defined above; and the pharmaceutically acceptable salts, individual isomers and mixtures of isomers thereof.

Preferred Formula I compounds include compounds wherein A is R₁₃-X₁₋, wherein R₁₃ is hydrogen, alkyloxycarbonylalkanoyl of overall 3 to 10 carbon atoms, (C₁₋₉)alkoxycarbonyl, (C₂₋₁₀)alkanoyl (optionally substituted with a radical selected from carboxy, (C₁₋₉)alkyloxycarbonyl and hetero(C₄₋₈)cycloalkyl(C₂₋₁₀)alkanoylamino), (C₄₋₈)cycloalkylcarbonyl, hetero(C₄₋₈)cycloalkylcarbonyl (optionally substituted with a radical selected from hydroxy, (C₁₋₅)alkyl, hetero(C₄₋₈)cycloalkyl, (C₁₋₅)alkanoyl, (C₁₋₅)alkyloxycarbonyl, (C₆₋₁₀)aryl(C₁₋₅)alkyloxycarbonyl and hetero(C₄₋₈)cycloalkylcarbonyl), (C₆₋₁₀)aryl(C₁₋₅)alkyloxycarbonyl, carbamoyl, (C₁₋₅)alkylcarbamoyl, di(C₁₋₅)alkylcarbamoyl, (C₆₋₁₀)arylcaramoyl, (C₆₋₁₀)aryl(C₁₋₅)alkylcarbamoyl, (C₆₋₁₀)aryl(C₁₋₅)alkanoyl, (C₇₋₁₁)aroyl, (C₁₋₅)alkylsulfonyl, di(C₁₋₅)alkylsulfamoyl, (C₆₋₁₀)arylsulfonyl, (C₆₋₁₀)aryl(C₁₋₅)alkylsulfonyl or hetero(C₄₋₈)arylsulfonyl; and X₁ is a divalent radical of Formula (a), wherein n is 0 to 5; X₁-X₂ represents a linkage selected from -C(O)NR₁₄-; Y is -N(R₁₄)-; Z is -CH(R₁₆)-; R₁₆ is hydrogen or as defined below; and R⁸ is (C₃₋₇)cycloalkyl, (C₃₋₇)cycloalkyl(C₁₋₅)alkyl, pyridyl, thienyl, furyl, imidazolyl, indolyl, pyridyl(C₁₋₆)alkyl, thienyl(C₁₋₆)alkyl, furyl(C₁₋₆)alkyl, imidazolyl(C₁₋₆)alkyl, indolyl(C₁₋₆)alkyl, (C₁₋₅)alkyl (optionally substituted with a radical selected from mercapto, carboxy, amino, methylthio, methylsulfonyl, carbamoyl, dimethylcarbamoyl, guanidino and hydroxy, or a protected derivative thereof), a group selected from phenyl, naphthyl, phenyl(C₁₋₆)alkyl, naphthyl(C₁₋₆)alkyl, (which group is optionally substituted at its aryl ring with one to three radicals selected from amino, hydroxy, chloro, bromo, fluoro, iodo, methyl, trifluoromethyl, methoxy and

phenyl, or a protected derivative thereof) or together with an adjacent R₁₄ forms a
divalent radical selected from (C₃₋₄)methylene and 1,2-phenylenedimethylene (which
radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo);
X is -S(O)₂R₉, wherein R₉ is (C₁₋₅)alkyl (optionally substituted with one or two radicals
selected from amino, chloro, bromo, fluoro, hydroxy and methoxy, or a protected
derivative thereof), perhalo(C₁₋₅)alkylsulfonyl, (C₃₋₇)cycloalkyl, (C₃₋₇)cycloalkyl(C₁₋₅)alkyl
or a group selected from phenyl, pentafluorophenyl, naphthyl and phenyl(C₁₋₅)alkyl
(which group is optionally substituted at its aryl ring with one to two radicals selected
from amino, chloro, bromo, fluoro, hydroxy, methoxy and optionally halo-substituted
methyl, or a protected derivative thereof);
R₁ is hydrogen and R₂ is R₁₆, as defined above; and
R₃ and R₄ are each hydrogen or together form optionally substituted ethylene.

Further preferred compounds of this embodiment include n is 0 to 2; R₂ is R₁₆, as
defined below; R₃ and R₄ together form optionally substituted ethylene; R₁₃ is
hydrogen, (C₄₋₆)alkoxycarbonyl, (C₂₋₆)alkanoyl (optionally substituted with a radical
selected from carboxy, (C₁₋₅)alkyloxycarbonyl and hetero(C₄₋₆)cycloalkyl-
(C₄₋₆)alkanoylamino), -C(O)NR₁₀R₁₁, wherein R₁₀ and R₁₁, together form
aza(C₂₋₆)methylen, oxa(C₂₋₆)methylen or (C₃₋₇)methylen, (C₄₋₆)cycloalkylcarbonyl,
benzyloxycarbonyl, acetyl, benzoyl or dimethylsulfamoyl; R₈ is (C₅₋₆)cycloalkyl,
(C₅₋₆)cycloalkylmethyl, 3-pyridyl, 2-thienyl, 2-furyl, 4-imidazolyl, 3-indolyl,
3-pyridylmethyl, 2-thienylmethyl, 2-furylmethyl, 4-imidazolylmethyl, 3-indolylmethyl;
methoxy, acetoxy, (C₁₋₅)alkyl (optionally substituted with a radical selected from
mercapto, carboxy, amino, methylthio, methylsulfonyl, carbamoyl, dimethylcarbamoyl,
guanidino and hydroxy, or a protected derivative thereof), a group selected from
phenyl, 1-naphthyl, 2-naphthyl, benzyl, 1-naphthylmethyl, 2-naphthylmethyl and
2-phenylethyl (which group is optionally substituted at its aryl ring with one radical
selected from hydroxy, amino, chloro, bromo and fluoro, or a protected form thereof) or
together with an adjacent R₁₄ forms a divalent radical selected from (C₃₋₄)methylene
and 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or
a protected derivative thereof, or oxo); and R₉ is (C₁₋₅)alkyl (optionally substituted with
one or two radicals selected from amino, chloro, bromo, fluoro and hydroxy, or a
protected derivative thereof), perfluoro(C₁₋₅)alkyl, (C₅₋₆)cycloalkyl, (C₅₋₆)cycloalkylmethyl
or a group selected from phenyl, naphthyl and benzyl (which group is optionally

substituted with one radical selected from amino hydroxy, chloro, bromo or fluoro, or a protected derivative thereof).

Particularly preferred compounds of this embodiment include n is 0 to 1; R₂ is butyl, 2-phenylethyl, 2-methylsulfonylethyl, 2-tert-butoxycarbonylethyl,

- 5 2-tert-butoxycarbonylmethyl, 4-tert-butoxycarbonylaminobutyl, 4-benzoylaminobutyl or benzylloxymethyl; R₁₃ is hydrogen, tert-butoxycarbonyl, benzylloxycarbonyl, acetyl, 3-carboxypropionyl, 3-methoxycarbonylpropionyl, biotinylaminohexanoyl, phenylacetyl, benzoyl, dimethylsulfamoyl, benzylsulfonyl, 1-piperizinylcarbonyl, 4-methyl-1-piperazinylcarbonyl or 4-morpholinylcarbonyl; R₁₆ is 3-pyridylmethyl,
- 10 2-thienylmethyl, 2-furylmethyl, 4-imidazolylmethyl, 3-indolylmethyl, (C₁₋₅)alkyl (optionally substituted with a radical selected from mercapto, carboxy, amino, methylthio, methylsulfonyl, carbamoyl, dimethylcarbamoyl, guanidino and hydroxy, or a protected derivative thereof), a group selected from benzyl, 1-naphthylmethyl, 2-naphthylmethyl and 2-phenylethyl (which group is optionally substituted at its aryl ring with one radical selected from hydroxy, amino, chloro, bromo and fluoro, or a protected form thereof) or together with an adjacent R₃ or R₁₃ forms a divalent radical selected from (C₃₋₄)methylene and 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo); and R₉ is 15 methyl, trifluoromethyl, optionally substituted phenyl, 2-naphthyl or 2-phenylethyl.
- 15 20 Especially preferred embodiments include n is 0; R₂ is butyl, 2-phenylethyl or 2-methylsulfonylethyl; R₁₃ is hydrogen, tert-butoxycarbonyl, benzylloxycarbonyl, biotinylaminohexanoyl, benzoyl, 1-piperizinylcarbonyl, 4-methyl-1-piperazinylcarbonyl or 4-morpholinylcarbonyl; R₁₆ is (C₁₋₅)alkyl, optionally substituted benzyl, 1-naphthylmethyl, 2-naphthylmethyl, 3-pyridinylmethyl or 2-methylsulfonylethyl; and R₉ is phenyl, 1-naphthyl or 2-phenylethyl.
- 25

- As are depicted in Figure 1, preferred inhibitors of the invention include (abbreviation first): Mu-Phe-retro-(D,L)-PheSO₂Ph: 2-benzyl-4-(morpholinocarbonylphenylalanyl)-1-phenylsulfonylethylenediamine; Mu-Phe-retro-(D,L)-LeuSO₂Ph: 2-isobutyl-4-(morpholinocarbonylphenylalanyl)-1-phenylsulfonylethylenediamine;
- 30 Mu-Tyr-retro-(D,L)-LeuSO₂Ph: 2-isobutyl-4-(morpholinocarbonyltyrosyl)-1-phenylsulfonylethylenediamine; Mu-Phe-retro-(D,L)-HphSO₂Ph: 4-(morpholinocarbonylphenylalanyl)-2-phenethyl-1-phenylsulfonylethylenediamine; Mu-

Tyr-retro-(D,L)Hph-SO₂Ph: 4-(morpholinecarbonyltyrosyl)-2-phenethyl-1-phenylsulfonylethylenediamine; Mu-Tyr-retro-(D,L)-NleSO₂Ph: 2-butyl-4-(morpholinecarbonyltyrosyl)-1-phenylsulfonylethylenediamine; Mu-Np2-retro-(D,L)-NleSO₂Ph: 2-butyl-4-(morpholinecarbonyl-2-naphthylalanyl)-1-phenylsulfonylethylenediamine; Boc-Np2-retro-(D,L)-NleSO₂Ph: 4-(tert-butoxycarbonyl-2-naphthylalanyl)-2-butyl-1-phenylsulfonylethylenediamine; Piv-Np2-retro-(D,L)-NleSO₂Ph: 2-butyl-4-(pivaloyl-2-naphthylalanyl)-1-phenylsulfonylethylenediamine; Mu-Np2-retro-(D,L)-HphSO₂Ph: 4-(morpholinecarbonyl-2-naphthylalanyl)-2-phenethyl-1-phenylsulfonylethylenediamine; Boc-Np2-retro-(D,L)-HphSO₂Ph: 4-(tert-butoxycarbonyl-2-naphthylalanyl)-2-phenethyl-1-phenylsulfonylethylenediamine; Mu-Phe-pip-retro-(D,L)-LeuSO₂Ph: 2-isobutyl-4-(morpholinecarbonylphenylalanyl)-1-phenylsulfonyl-1,4-piperazine; Mu-Phe-pip-retro-(D,L)-HphSO₂Ph: 4-(morpholinecarbonylphenylalanyl)-2-phenethyl-1-phenylsulfonyl-1,4-piperazine; Z-Np2-retro-(D,L)-NleSO₂2Np: 4-(benzyloxycarbonyl-2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-ethylenediamine; Bzac-Np2-retro-(D,L)-NleSO₂2Np: 4-(benzylaminocarbonyl-2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-ethylenediamine; BzISO₂-Np2-retro-(D,L)-NleSO₂2Np: 4-(benzylsulfonyl-2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine; Mu-Np2-pip-retro-(D,L)-NleSO₂2Np: 2-butyl-4-(morpholinecarbonyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; MeOSuc-Np2-pip-retro-(D,L)-NleSO₂2Np: 2-butyl-4-(methoxycarbonylpropionyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Z-Np2-pip-retro-(D,L)-NleSO₂2Np: 4-(benzyloxycarbonyl-2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine; Bzac-Np2-pip-retro-(D,L)-NleSO₂2Np: 4-(benzylaminocarbonyl-2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine; BzISO₂-Np2-pip-retro-(D,L)-NleSO₂2Np: 4-(benzylsulfonyl-2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine; PhSO₂-Np2-pip-retro-(D,L)-NleSO₂2Np: 2-butyl-1-(2-naphthylsulfonyl)-4-(phenylsulfonyl-2-naphthylalanyl)-1,4-piperazine; tBac-Np2-pip-retro-(D,L)-NleSO₂2Np: 2-butyl-4-(tert-butylaminocarbonyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Mu-Phe-pip-GlySO₂Ph: 4-(morpholinecarbonylphenylalanyl)-1-phenylsulfonyl-1,4-piperazine; Z-Np2-pip-GlySO₂2Np: 4-(benzyloxycarbonyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Phac-Np2-pip-GlySO₂2Np: 1-(2-naphthylsulfonyl)-4-(phenylacetyl-2-naphthylalanyl)-1,4-piperazine; t-Buac-Np2-pip-GlySO₂2Np: 4-(tert-butylacetyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Boc-Np2-pip-GlySO₂2Np: 4-(tert-butoxycarbonyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Z-Np2-pip-GlySO₂Bzl: 4-

(benzyloxycarbonyl-2-naphthylalanyl)-1-benzylsulfonyl-1,4-piperazine; and Phac-Np2-pip-GlySO₂Bzl: 1-benzylsulfonyl-4-(phenylacetyl-2-naphthylalanyl)-1,4-piperazine.

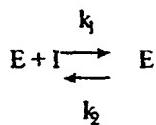
- Additionally preferred compounds (not pictured in Figure 1) include: Z-leu-pip-GlySO₂Np2: 4-(benzyloxycarbonylleucyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Z-alapip-glySO₂Np2: 4-(benzyloxycarbonylalanyl)-1-(2-naphthylsulfonyl)-1,4-piperazine;
- 5 Np1SO₂-leu-pip-GlySO₂Np2: 4-(1-naphthylsulfonylleucyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; Np2SO₂-leu-pip-GlySO₂Np2: 4-(2-naphthylsulfonylleucyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; PP-leu-pip-GlySO₂Np2: 4-[4-(1-piperidyl)-1-piperidoylleucyl]-1-(2-naphthylsulfonyl)-1,4-piperazine; Np2-arg-pip-GlyZ: 4-(2-
- 10 naphthylsulfonylarginyl)-1-benzyloxycarbonyl-1,4-piperazine; Np2-tyr-Z-le-pip-GlySO₂Np2: 4-(2-naphthylsulfonyltyrosyl)-1-(2-naphthylsulfonyl)-1,4-piperazine; MP-leu-pip-retro-(D,L)-HphSO₂Np2: 4-(4-methylpiperazinoylleucyl)-1-(2-naphthylsulfonyl)-2-(2-phenylethyl)-1,4-piperazine; tBoc-nap2-pip-Z-le-pip-GlySO₂Np2: 4-[isobutoxycarbonyl-(2-naphthyl)alanyl]-1-(2-naphthylsulfonyl)-1,4-piperazine; PM-nap2-
- 15 15 pip-GlySO₂Np2: 4-[4-piperidylmethoxycarbonyl-(2-naphthyl)alanyl]-1-(2-naphthylsulfonyl)-1,4-piperazine; BI-nap2-pip-SO₂Np2: 4-[6-benzimidazoloyl-(2-naphthyl)alanyl]-1-(2-naphthylsulfonyl)-1,4-piperazine; and MP-leu-pip-retro-(D,L)-NleSO₂Np2: 2-butyl-4-(4-methylpiperazinoylleucyl)-1-(2-naphthylsulfonyl)-1,4-piperazine.
- 20 Additional preferred compounds of the invention include: tBoc-leu-pip-(D,L)-(MeO)(Me)NCOGlySO₂Np2: 4-(tert-butoxycarbonylleucyl)-3-(N-methoxy)(N-methyl)carbamoyl-1-(2-naphthylsulfonyl)-1,4-piperazine; Z-leu-pip-retro-(D,L)-MeOCOGlySO₂Np2: 4-(benzyloxycarbonylleucyl)-2-methoxycarbonyl-1-(2-naphthylsulfonyl)-1,4-piperazine; Z-leu-pip-retro-(D,L)-HCOGlySO₂Np2: 4-(benzyloxycarbonylleucyl)-2-carboxy-1-(2-naphthylsulfonyl)-1,4-piperazine; Z-leu-pip-retro-(D,L)-EtACOGlySO₂Np2: 4-(benzyloxycarbonylleucyl)-2-ethylcarbamoyl-1-(2-naphthylsulfonyl)-1,4-piperazine; and Z-leu-pip-retro-(D,L)-AEtACOGlySO₂Np2: 4-(benzyloxycarbonylleucyl)-2-(2-aminoethylcarbamoyl)-1-(2-naphthylsulfonyl)-1,4-piperazine.
- 25 30 In some embodiments, the stereochemistry of the amino acid side chains at positions R₁, R₂, R₅ and R₆ is important. That is, the amino acid side chains may be in either the (D) or (R) configuration, or the (L) or (S) configuration.

In a preferred embodiment, the dissociation constant for inhibition of a protease with the inhibitor, generally referred to by those in the art as K_i , is at most about 100 μM . By the term "binding constant" or "dissociation constant" or grammatical equivalents herein is meant the equilibrium dissociation constant for the reversible association of inhibitor with enzyme. The dissociation constants are defined and determined as below.

The determination of dissociation constants is known in the art. For example, for reversible inhibition reactions such as those of the present invention, the reaction scheme is as follows:

10

Equation 1



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The enzyme and the inhibitor combine to give an enzyme-inhibitor complex, $E \cdot I$. This step is assumed to be rapid and reversible, with no chemical changes taking place; the enzyme and the inhibitor are held together by non-covalent forces. In this reaction, k_1 is the second order rate constant for the formation of the $E \cdot I$ reversible complex. k_2 is the first order rate constant for the disassociation of the reversible $E \cdot I$ complex. In this reaction, $K_i = k_2/k_1$.

The measurement of the equilibrium constant K_i proceeds according to techniques well known in the art, as described in the examples. For example, assays generally use synthetic chromogenic or fluorogenic substrates.

20

The respective K_i values may be estimated using the Dixon plot as described by Irwin Segel in Enzyme Kinetics: Behavior and analysis of rapid equilibrium and steady-state enzyme systems, 1975, Wiley-Interscience Publication, John Wiley & Sons, New York, or for competitive binding inhibitors from the following calculation:

Equation 2

25

$$1 - (v/v_o) = [I]/([I] + K_i(1 + ([S]/K_M)))$$

wherein

v_0 is the rate of substrate hydrolysis in the absence of inhibitor, and v_i is the rate in the presence of competitive inhibitor.

It is to be understood that dissociation constants are a particularly useful way of quantifying the efficiency of an enzyme with a particular substrate or inhibitor, and are frequently used in the art as such. If an inhibitor exhibits a very low K_i , it is an efficient inhibitor. Accordingly, the cysteine protease inhibitors of the present invention have dissociation constants, K_i , of at most about 100 μM . Preferred embodiments have inhibitors that exhibit dissociation constants of at most about 10 μM , at most about 1 μM , with the most preferred embodiments having dissociation constants of at most 5 about 100 nM.

In the preferred embodiment, the cysteine protease inhibitors are chiral. By the term "chiral" or grammatical equivalents herein is meant a compound that exhibits asymmetry. That is, the chiral compound is not identical with its mirror image. Thus in the preferred embodiment, the compounds of the present invention are pure 10 diasteromers. Chiral compounds, and particularly chiral cysteine protease inhibitors, are useful in the present invention because biological systems, and enzymes in particular, are stereospecific, preferring the (S) or L-form of amino acids. Thus in the preferred embodiment, the A and X groups of the cysteine protease inhibitors of the present invention will have amino acid side chains in the (S) or L-configuration, 15 although some inhibitors may utilize amino acid side chains in the (R) or D-configuration.

In alternative embodiments, the compositions of the present invention are not pure epimers, but are mixtures that contain both epimers.

The synthesis of the cysteine protease inhibitors of the present invention proceeds as 20 follows.

Synthesis of cysteine protease inhibitors with A and X groups linked via substituted ethylenediamine linkages is depicted generally in Figures 2 and 3. For unsubstituted ethylenediamine linkages, R₁ and R₂ are hydrogen. Figure 3 depicts the R₁ synthesis, 25 Figure 4 depicts the R₂ synthesis. Both schemes are depicted using a peptidyl group as a representative "A" moiety and a sulfonyl group as a representative "X" moiety;

however, those in the art will appreciate that these schemes may be used to synthesize cysteine protease inhibitors with other A and X groups.

Synthesis of cysteine protease inhibitors with R₂ and R₅ substituted piperazine as the linkage is depicted generally in Figure 4. For unsubstituted piperazine, R₂ and R₅ are 5 hydrogen. As above, a peptidyl group is shown as a representative "A" group and a sulfonyl group as the representative "X" group; as above, those in the art will appreciate that this scheme may be used to synthesize cysteine protease inhibitors with other A and X groups.

Synthesis of cysteine protease inhibitors with R₁ and R₆ substituted piperazine as the 10 linkage is depicted generally in Figure 5. For unsubstituted piperazine, R₁ and R₆ are hydrogen. As above, a peptidyl group is shown as a representative "A" group and a sulfonyl group as the representative "X" group; as above, those in the art will appreciate that this scheme may be used to synthesize cysteine protease inhibitors with other A and X groups.

15 Intermediates useful for the preparation of compounds of the invention in which R₁ or R₂ is carboxy, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkoxy(alkyl)carbamoyl or aminoalkylcarbamoyl are commercially available or can be readily prepared by one of ordinary skill in the art. For example, reactive piperazine intermediates in which R₁ or R₂ forms an amide derivative can be prepared by acylation of an appropriate amine, or 20 protected derivative thereof, with an appropriate protected carboxylic acid (e.g. N-protected 2-piperazinecarboxylic acid) and then deprotecting. The protected piperazine carboxylic acid is prepared from commercially available starting material via standard protection chemistry.

As will be appreciated in the art, the above synthetic techniques may be used to 25 synthesize the cysteine protease inhibitors of the invention. Representative examples of such syntheses are outlined in the Examples.

In one embodiment, the cysteine protease inhibitors of the present invention are further purified if necessary after synthesis, for example to remove unreacted materials. For example, the cysteine protease inhibitors of the present invention may be crystallized,

or passed through chromatography columns using solvent mixtures to elute the pure inhibitors.

Once produced, the cysteine protease inhibitors of the present invention may be easily screened for their inhibitory effect. The inhibitor is first tested against the cysteine protease for which the targeting group of the inhibitor was chosen, as outlined above. Alternatively, many cysteine proteases and their corresponding chromogenic substrates are commercially available. Thus, a variety of cysteine proteases are routinely assayed with synthetic chromogenic substrates in the presence and absence of the cysteine protease inhibitor, to confirm the inhibitory action of the compound, using techniques well known in the art. The effective inhibitors are then subjected to kinetic analysis to calculate the K_i values, and the dissociation constants determined.

If a compound inhibits at least one cysteine protease, it is a cysteine protease inhibitor for the purposes of the invention. Preferred embodiments have inhibitors that exhibit the correct kinetic parameters against at least the targeted cysteine protease.

In some cases, the cysteine protease is not commercially available in a purified form. The cysteine protease inhibitors of the present invention may also be assayed for efficacy using biological assays. For example, the inhibitors may be added to cells or tissues that contain cysteine proteases, and the biological effects measured.

In one embodiment, the cysteine protease inhibitors of the present invention are synthesized or modified such that the in vivo and in vitro proteolytic degradation of the inhibitors is reduced or prevented. Generally, this is done through the incorporation of synthetic amino acids, derivatives, or substituents into the cysteine protease inhibitor. Preferably, only one non-naturally occurring amino acid or amino acid side chain is incorporated into the cysteine protease inhibitor, such that the targeting of the inhibitor to the enzyme is not significantly affected. However, some embodiments that use longer cysteine protease inhibitors containing a number of targeting residues may tolerate more than one synthetic derivative. In addition, non-naturally occurring amino acid substituents may be designed to mimic the binding of the naturally occurring side chain to the enzyme, such that more than one synthetic substituent is tolerated.

Alternatively, peptide isosteres are used to reduce or prevent inhibitor degradation.

In this embodiment, the resistance of the modified cysteine protease inhibitors may be tested against a variety of known commercially available proteases in vitro to determine their proteolytic stability. Promising candidates may then be routinely screened in animal models, for example using labelled inhibitors, to determine the in vivo stability and efficacy.

In one embodiment, the cysteine protease inhibitors of the present invention are labelled. By a "labelled cysteine protease inhibitor" herein is meant a cysteine protease inhibitor that has at least one element, isotope or chemical compound attached to enable the detection of the cysteine protease inhibitor or the cysteine protease inhibitor bound to a cysteine protease. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the cysteine protease inhibitor at any position. Examples of useful labels include ^{14}C , ^3H , biotin, and fluorescent labels as are well known in the art.

In the preferred embodiment, the cysteine protease inhibitors of the present invention are administered to a patient to treat cysteine protease-associated disorders. By "cysteine protease-associated disorders" or grammatical equivalents herein is meant pathological conditions associated with cysteine proteases. In some disorders, the condition is associated with increased levels of cysteine proteases; for example, arthritis, muscular dystrophy, inflammation, tumor invasion, and glomerulonephritis are all associated with increased levels of cysteine proteases. In other disorders or diseases, the condition is associated with the appearance of an extracellular cysteine protease activity that is not present in normal tissue. In other embodiments, a cysteine protease is associated with the ability of a pathogen, such as a virus, to infect or replicate in the host organism.

Specific examples of cysteine protease associated disorders or conditions include, but are not limited to, arthritis, muscular dystrophy, inflammation, tumor invasion, glomerulonephritis, malaria, Alzheimer's disease, disorders associated with autoimmune system breakdowns, periodontal disease, cancer metastasis, trauma, inflammation, gingivitis, leishmaniasis, filariasis, osteoporosis and osteoarthritis, and other bacterial and parasite-borne infections, and others outlined above.

In particular, disorders associated with interleukin 1 β converting enzyme (ICE) are included, as outlined above.

In this embodiment, a therapeutically effective dose of a cysteine protease inhibitor is administered to a patient. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the disorder to be treated and the amount of cysteine protease to be inhibited, and will be ascertainable by one skilled in the art using known techniques. In general, the cysteine protease inhibitors of the present invention are administered at about 1 to about 1000 mg per day. For example, as outlined above, some disorders are associated with increased levels of cysteine proteases. Due to the 1:1 stoichiometry of the inhibition reaction, the dose to be administered will be directly related to the amount of the excess cysteine protease. In addition, as is known in the art, adjustments for inhibitor degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the disease may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

A "patient" for the purposes of the present invention includes both humans and other animals and organisms. Thus the methods are applicable to both human therapy and veterinary applications. For example, the veterinary applications include, but are not limited to, canine, bovine, feline, porcine, caprine, equine, and ovine animals, as well as other domesticated animals including reptiles, such as iguanas, turtles and snakes, birds such as finches and members of the parrot family, lagomorphs such as rabbits, rodents such as rats, mice, guinea pigs and hamsters, amphibians, fish, and arthropods. Valuable non-domesticated animals, such as zoo animals, may also be treated. In the preferred embodiment the patient is a mammal, and in the most preferred embodiment the patient is human.

The administration of the cysteine protease inhibitors of the present invention can be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in

the treatment of wounds and inflammation, the cysteine protease inhibitors may be directly applied as a solution or spray.

The pharmaceutical compositions of the present invention comprise a cysteine protease inhibitor in a form suitable for administration to a patient. In the preferred embodiment, the pharmaceutical compositions are in a water soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All references cited herein are incorporated by reference.

EXAMPLES

Example 1

Synthesis of Cysteine Protease Inhibitor
with a carbamoyl peptidyl A group, a sulfonyl X group, and
ethylenediamine backbone

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Unless otherwise indicated, all reactions were performed under an inert atmosphere of argon at room temperature. THF was distilled from sodium benzophenone ketyl. All other solvents and commercially available material were used without further purification. Unless otherwise noted, all reagents were obtained from Aldrich, Inc.

10 (4-morpholinecarbonylphenylalanyl)-2-phenethyl-1-phenylsulfonyl
ethylenediamine (Mu-Phe-retro-(D,L)-Hph-SO₂Ph)

To a suspension of homophenylalanine (Synthetech) (7 g, 39.1 mmol) in distilled water (21.5 mL) was added a 2 M aqueous NaOH (21.5 mL). After 5 minutes, the suspension had cleared and phenylsulfonyl chloride (5.48 mL, 43 mmol) was added. 15 After 2 hours, the reaction mixture's pH was adjusted to 12 with 2 M aqueous NaOH (5 mL), and extracted with Et₂O (2 x 100 mL). The aqueous layer's pH was adjusted to 1 with 6 M HCl (10 mL), and the product was extracted with EtOAc (100 mL), dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The residue was triturated with Et₂O (2 x 100 mL), filtered, and pumped to dryness, giving 8.74 g 20 (70%) of phenylsulfonylhomophenylalanine.

To a solution of 4-morpholinecarbonylphenylalanine (Mu-PheOH, 0.14 g, 0.48 mmol, prepared according to the method described in Esser, R. et.al., Arthritis & Rheumatism (1994), 37, 236) in THF (10 mL) at -10°C were added 4-methylmorpholine (0.11 mL, 0.96 mmol), followed by isobutyl chloroformate (64 μL, 0.48 mmol). After 5 minute activation, 2-phenethyl-1-phenylsulfonylethylenediamine hydrochloride (0.15 g, 0.44 mmol, prepared by conversion of the N-phenylsulfonylhomophenylalanine to the amide via CDI/NH₃/THF reaction, then by reduction of the carbonyl amide to its corresponding amine with lithium aluminium hydride, followed by treatment with HCl in dioxane) was added. After 1 hour, the reaction mixture was diluted with CH₂Cl₂ (50 mL) and 25 washed with 1M HCl (50 mL) followed by saturated aqueous NaHCO₃ (50 mL), dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The 30

residue was diluted with CH_2Cl_2 (5 mL) and precipitated from hexane (200 mL), filtered, and dried in vacuo yielding 0.22 g (89%) of the desired product.

Thin-layer chromatography (TLC) was performed on each sample. Visualization was accomplished by means of a UV light at 254 nm, followed by ninhydrin, bromocresol green, or p-anisaldehyde stain. The retention factor (R_f) of the Mu-Phe-retro-(D,L)-Hph-SO₂Ph was 0.67 (10% MeOH/ CH_2Cl_2).

EXAMPLE 2

Synthesis of a Cysteine Protease Inhibitor with an acyl peptidyl A group, a sulfonyl X group, and an ethylenediamine backbone.

- 10 Synthesis of 2-butyl-4-(pivaloyl-2-naphthylalanyl)-1-phenylsulfonyl ethylenediamine (abbreviated Piv-Np2-retro-(D,L)-NleSO₂Ph). To a solution of HCl*norleucine methyl ester (4.87 g, 26.8 mmol) in THF (20 mL) were added triethylamine (8.22 mL, 59 mmol) and phenylsulfonyl chloride (3.76 mL, 30 mmol). After 16 hours, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with 1 M HCl, saturated aqueous NaHCO₃, dried over MgSO₄, and filtered. The solvent was removed under reduced pressure giving 6.04 g (79%) of N-phenylsulfonylnorleucine methyl ester.
- 15 To a solution of t-butoxycarbonyl-2-naphthylalanine (Synthetech) (0.59 g, 1.88 mmol) in THF (25 mL) at -10°C were added 4-methylmorpholine (0.41 mL, 3.8 mmol), followed by isobutyl chloroformate (0.25 mL, 1.88 mmol). After 5 minute activation, 2-(D,L)-butyl-1-phenyl sulfonylethylenediamine hydrochloride (0.5 g, 1.7 mmol, prepared by conversion of the N-phenylsulfonylnorleucine methyl ester to its corresponding acid, by aqueous sodium hydroxide treatment; amide formation, CDI/NH₃; reduction of the carbonyl amide to its corresponding amine; followed by HCl dioxane treatment) was added. After 1 hour, the reaction was diluted with EtOAc (100 mL) and washed with 1 M HCl (50 mL), saturated aqueous NaHCO₃ (50 mL), dried over MgSO₄, and filtered. The solvent was removed under reduced pressure, triturated with hexane (15 mL) and dried in vacuo, giving 0.40 g (76%) of Boc-Np2-retro-(D,L)-NleSO₂Ph.

To a solution of HCl*^Np2-retro-(D,L)-NleSO₂Ph (0.97 g, 1.98 mmol, prepared by conversion of Boc-Np2-retro-(D,L)-NleSO₂Ph to HCl*^Np2-retro-(D,L)-NleSO₂Ph via HCl/dioxane) in THF (25 mL) at -10° C were added pivaloyl chloride (0.27 mL, 2.2 mmol) and 4-methylmorpholine (0.48 mL, 4.4 mmol). After 1 hour, the reaction mixture was diluted with CH₂Cl₂ (100 mL) and washed with 1 M HCl (100 mL), saturated aqueous NaHCO₃ (100 mL), dried over MgSO₄, and filtered. The solvent was removed under reduced pressure yielding 0.66 g (66%) of Piv-Np2-retro-(D,L)-NleSO₂Ph TLC: R_f = 0.3 (20% EtOAc/CH₂Cl₂).

Example #3

10 Synthesis of a Cysteine Protease Inhibitor containing a sulfonyl peptidyl A group, a sulfonyl X group, and a piperazine backbone.

Synthesis of 2-butyl-1-(2-naphthylsulfonyl)-4-(phenylsulfonyl-2-naphthylalanyl)-1,4-piperazine, abbreviated PhSO₂-Np2-pip-retro-(D,L)-NleSO₂2Np.

15 To a solution, Z-Nle-OH (10 g, 38 mmol) in THF (100 mL) at -10°C were added pyridine (6.7 mL, 83 mmol), and thionyl chloride (3.02 mL, 42 mmol). After 30 minutes, triethylamine (11.6 mL, 83 mmol) and N-benzylglycine ethyl ester (5.4 mL, 42 mmol) were added. After 2 hours, the reaction mixture was diluted with EtOAc (200 mL) and washed with 1 M HCl (150 mL), saturated aqueous NaHCO₃ (150 mL), dried over MgSO₄, and filtered. The solvent was removed under reduced pressure, yielding 16.61 (100%) of Z-norleucyl-N-benzylglycine ethyl ester.

20 To a solution of HBr*norleucyl-N-benzylglycine ethyl ester (14.6 g, 38 mmol, prepared by conversion of the Z-norleucyl-N-benzylglycine ethyl ester to its HBr salt with 30% HBr in AcOH.) in EtOH (50 mL) was added triethylamine (13.2 mL, 94 mmol). After 2 hours the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with 1 M HCl (100 mL), saturated aqueous NaHCO₃ (100 mL), dried over MgSO₄, and filtered. Diluted with Et₂O (300 mL) and placed at 0° C for 2 hours. The solution was filtered and the solid was dried in vacuo yielding 6.3 g (64%) 1-benzyl-3-butylidiketo-1,4-piperazine.

25 To a solution of 2(HCl)*1-benzyl-3-butyl-1,4-piperazine (2.2 g, 8.2 mmol, prepared by conversion of 1-benzyl-3-butylidiketo-1,4-piperazine to 4-benzyl-3-butyl-1,4-piperazine

- via reduction with lithium aluminum hydride followed by HCl/dioxane treatment) in THF (50 mL) were added, BSA (4.4 mL, 18 mmol), triethylamine (2.5 mL, 18 mmol), and 2-naphthylsulfonyl chloride (2 g, 9 mmol). After 16 hours, the reaction mixture was diluted with EtOAc (100 mL) and washed with 1 M HCl (100 mL), saturated aqueous NaHCO₃ (100 mL), dried over MgSO₄, and filtered. The solvent was removed under reduced pressure and the residue was diluted with CH₂Cl₂ (100 mL). A mixture of MgSO₄/activated carbon/silica (1:1:1, 5 g) was added to the solution, heated to reflux for 5 minutes, and filtered. The solvent was removed under reduced pressure yielding 2.7 g (80%) of 4-benzyl-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine.
- 5
- 10 To a solution of Z-(2-naphthylalanine) (2.1 g, 5.9 mmol, prepared by conversion of 2-naphthylalanine (Synthetech) to its Z-derivative with benzyloxycarbonyl chloride.) in THF (50 mL) at -10° C were added pyridine (0.96 mL, 12 mmol), thionyl chloride (0.43 mL, 5.9 mmol). After 30 minutes, a solution of 2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine hydrochloride (2.4 g, 5.4 mmol, prepared by conversion of
- 15 1-benzyl-3-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine to 2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine with H₂/5% Pd/C/IPA (isopropanol) in a Parr apparatus, and followed by HCl/dioxane treatment) BSA (1.5 mL, 5.9 mmol), and triethylamine (1.7 mL, 12 mmol) in CH₂Cl₂ (50 mL) was added. After 1 hour, the reaction mixture was diluted in CH₂Cl₂ (100 mL) and washed with 1 M HCl (100 mL), saturated aqueous NaHCO₃, dried over MgSO₄, and filtered. The solvent was removed under reduced pressure, diluted with Et₂O (10 mL), precipitated from hexane (100 mL), filtered, and dried in vacuo giving 1.7 g (43%) of 4-(Z-(2-naphthyl-alanyl))-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine.
- 20
- 25 To a solution of 4-(2-naphthylalanyl)-2-butyl-1-(2-naphthylsulfonyl)-1,4-piperazine hydrobromide (0.2 g, 0.35 mmol) prepared from the Z derivative via 30% HBr in AcOH) in THF (20 mL) at -10° C were added BSA (0.19 mL, 0.76 mmol), phenylsulfonyl chloride (50 μL, 0.38 mmol), and triethylamine (0.11 mL, 0.76 mmol). After 1 hour, the reaction mixture was diluted with EtOAc (50 mL), washed with 1 M HCl (50 mL), saturated aqueous NaHCO₃ (100 mL), dried over MgSO₄, and filtered. The solvent was removed under reduced pressure, and the residue was diluted with CH₂Cl₂ (5 mL), precipitated with diisopropyl ether (50 mL), filtered, and dried in vacuo giving 0.1 g (43%) of final product. TLC: R_f=0.78 (50% EtOAc/CH₂Cl₂)
- 30

Synthesis of Cysteine Protease Inhibitor Containing
a alkyloxycarbonyl peptidyl A group, a sulfonyl X group, and a
piperazine backbone.

5 Synthesis of 4-(benzyloxycarbonyl-2-naphthylalanyl)-1-(2-naphthylsulfonyl)
-1,4-piperazine, abbreviated Z-Np2-pip-GlySO₂Np.

To a suspension of piperazine (3.4 g, 40 mmol) in the THF (100 mL) at 0° C were
added BSA (9.8 mL, 40 mmol) and 2-naphthylsulfonyl chloride (3 g, 13 mmol). After
30 minutes, the reaction was filtered and the solvent was removed under reduced
pressure. The residue was diluted in CH₂Cl₂ (100 mL), washed with saturated
10 aqueous NaHCO₃ (100 mL), dried over MgSO₄, and filtered. The solvent was
removed under reduced pressure, triturated with Et₂O (20 mL)/hexane (20 mL), and
dried in vacuo giving 3.4 g (92%) 1-(2-naphthylsulfonyl)-1,4-piperazine.

15 To a solution of Z-(2-naphthylalanine) (2.1 g, 5.8 mmol) in THF (50 mL) at 0° C were
added pyridine (0.97 mL, 11 mmol) and thionyl chloride (0.43 mL, 6 mmol). After 30
minutes, 1-(2-naphthylsulfonyl)-1,4-piperazine (1.5 g, 5.4 mmol) and triethylamine (1.7
mL, 12 mmol) were added. After 1 hour the reaction was diluted with EtOAc (100 mL),
washed with 1 M HCl (100 mL), saturated aqueous NaHCO₃ (100 mL), dried over
20 MgSO₄, filtered. The solvent was removed under reduced pressure and diluted with
CH₂Cl₂ (20 mL), precipitated from hexane (200 mL), filtered, and dried in vacuo giving
1.8 g (55%) of the desired product. TLC: R_f=0.39 (50% EtOAc/hexane).

Example #5

25 Synthesis of Cysteine Protease Inhibitor Containing
an acyl peptidyl A group, a sulfonyl X group,
and a piperazine backbone.

Synthesis of 1-benzylsulfonyl-4-(phenylacetyl-(2-naphthyl alanyl))-1,4-piperazine,
abbreviated Phac-Np2-pip-GlySO₂Bzl.

To a suspension of piperazine (4 g, 46 mmol) in THF (100 mL) at -10° C were added
BSA (11.5 mL, 46 mmol) and α-toluenesulfonyl chloride (3 g, 15 mmol). After 1 hour
30 the reaction was filtered, diluted with EtOAc (100 mL), washed with saturated aqueous
NaHCO₃, dried over MgSO₄, and filtered. The solvent was removed under reduced

concentrations of inhibitors was noted. The assay was linear throughout the range observed. Duplicate runs were measured.

Conditions for cathepsin L: 50 mM acetate, pH 5.5, 2.5 mM EDTA, 2.5 mM DTT.

Substrate: [Z-Phe-Arg-AMC] = 5 μ M (K_m = 2 μ M). The assay at 25° was started by 5 the addition of cat L (final concentration approx 1 nM) and the increase in fluorescence at 450 nm with excitation at 380 nm was followed over 2 min. The depression in the rate of substrate hydrolysis following addition of varying concentrations of inhibitors was noted. The assay was linear throughout the range observed. Duplicate runs were measured.

10 Conditions for cathepsin S: 50 mM phosphate, pH 6.5, 2.5 mM EDTA, 2.5 mM DTT.

Substrate: [Z-Val-Val-Arg-AMC] = 10 μ M (K_m = 18 μ M). The assay at 25° was started by the addition of cat S (final concentration approx. 30 pM) and the increase in fluorescence at 450 nm with excitation at 380 nm was followed over 2 min. The depression in the rate of substrate hydrolysis following addition of varying 15 concentrations of inhibitors was noted. The assay was linear throughout the range observed. Duplicate runs were measured.

Conditions for cruzain were the same as for cathepsin L with the exception that the K_m for the substrate was 1 μ M.

20 The respective K_i values were estimated by using the Dixon plot as described by Irwin Segel in Enzyme Kinetics: Behavior and analysis of rapid equilibrium and steady-state enzyme systems, 1975, Wiley-Interscience Publication, John Wiley & Sons, New York, or for competitive binding inhibitors from the following calculation:

$$1 - \frac{v_i}{v_o} = \frac{[I]}{[I] + K_i (1 + \frac{[S]}{K_m})}$$

v_o is the rate of substrate hydrolysis in the absence of inhibitor, whereas v_i is the rate in the presence of competitive inhibitor.

pressur , and the residue was triturated with Et₂O (20 mL)/hexane (20 mL), and pumped to a solid giving 2.5 g (67%) of 1-benzylsulfonyl-1,4-piperazine.

To a solution of Z-(2-naphthylalanine) (0.81 g, 6.9 mmol) in THF (50 mL) were added pyridine (1.12 mL, 13.7 mmol) and thionyl chloride (0.5 mL, 6.9 mmol). After 30
5 minutes, a solution of 1-benzylsulfonyl-1,4-piperazine (1.5 g, 6.2 mmol), BSA (3.4 mL,
13.7 mmol), and triethylamine (1.9 mL, 13.7 mmol) in THF (50 mL) was added. After 2
hours, the reaction mixture was diluted with EtOAc (200 mL), washed with 1 M HCl
(200 mL), saturated aqueous NaHCO₃ (200 mL) dried over MgSO₄, and filtered. The
solvent was removed under reduced pressure. The residue was dissolved in CH₂Cl₂
10 (30 mL), precipitated from hexane (200 mL), filtered, and dried in vacuo giving 1.5 g
(70%) of 4-(benzyloxycarbonyl-(2-naphthylalanyl)-1-benzylsulfonyl-1,4-piperazine,
Z-Np2-pip-GlySO₂Bzl.

To a solution of 4-(2-naphthylalanyl)-1-benzylsulfonyl-1,4-piperazine (0.40 g, 0.77 mmol,
prepared by conversion of Z-Np2-pip-GlySO₂Bzl to its HBr salt with 30% HBr in AcOH)
15 in THF (50 mL) at -10° C were added phenylacetyl chloride (0.11 mL, 0.84 mmol) and
triethylamine (0.23 mL, 1.7 mmol). After 1 hour, the reaction was diluted with EtOAc
(100 mL), washed with 1 M HCl (100 mL), saturated aqueous NaHCO₃ (100 mL), dried
over MgSO₄, and filtered. The solvent was removed under reduced pressure, and the
residue was dissolved in CH₂Cl₂ (10 mL), precipitated from hexane (100 mL), filtered,
20 and dried in vacuo yielding 0.28 g (65%) of the desired product. TLC: R_f=0.39 (20%
EtOAc/CH₂Cl₂)

The remainder of the compounds depicted in Figure 1 were synthesized using the
above techniques.

Example 6

Inhibition Kinetics

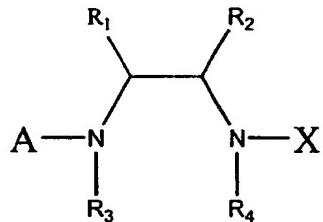
Conditions for cathepsin B: 50 mM phosphate, pH 6.0, 2.5 mM EDTA, 2.5 mM DTT.
Substrate: [Z-Arg-Arg-AMC] = 50 μM (K_m = 190 μM). The assay at 25° was started
by the addition of cat B (final concentration approx 10 nM) and the increase in
fluorescence at 450 nm with excitation at 380 nm was followed over 2 min. The
30 depression in the rate of substrate hydrolysis following addition of varying

CLAIMS

1. A reversible cysteine protease inhibitor comprising two N-substituents linked via an ethylenediamine or a substituted ethylenediamine, wherein the dissociation constant for inhibition, K_i , of a protease with the inhibitor, is no greater than about 100 μM , and

5 wherein said N-substituents are selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl.

2. A reversible cysteine protease inhibitor having the formula comprising:



10 wherein

A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl;

15 R₁ is (a) an amino acid side chain or (b) hydrogen;

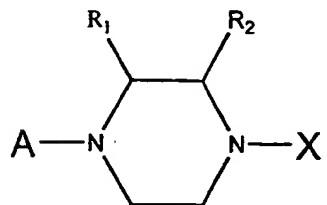
R₂ is (a) an amino acid side chain or (b) hydrogen, wherein either (1) both R₁ and R₂ are hydrogen, or (2) one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen; and

20 R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene;

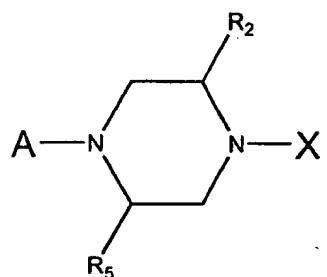
wherein the dissociation constant for inhibition, K_i , of a protease with the inhibitor, is no greater than about 100 μM .

3. A reversible cysteine protease inhibitor according to claim 2 wherein R₂ is an amino acid side chain.

4. A reversible cysteine protease inhibitor according to claim 2 having the formula comprising:



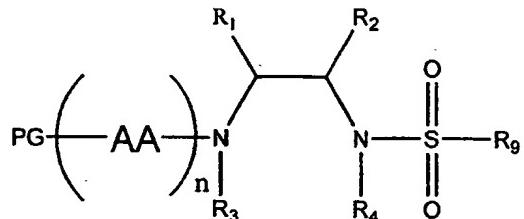
5. A reversible cysteine protease inhibitor according to claim 2 having the formula comprising:



5 wherein:

R₅ is (a) an amino acid side chain or (b) hydrogen, wherein either both R₂ and R₅ are hydrogen, or one of R₂ or R₅ is an amino acid side chain and the other one of R₂ and R₅ is hydrogen.

6. A cysteine protease inhibitor according to claim 2 having the formula comprising:



10 wherein

n is from 1 to 10;

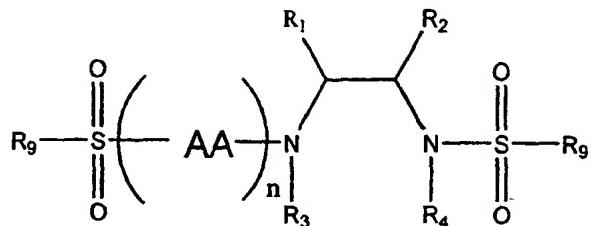
PG is a protecting group;

AA is an amino acid;

R is an amino acid side chain; and

15 -SO₂R₉ is a sulfonyl moiety.

7. A cysteine protease inhibitor according to claim 2 having a formula comprising:



wherein

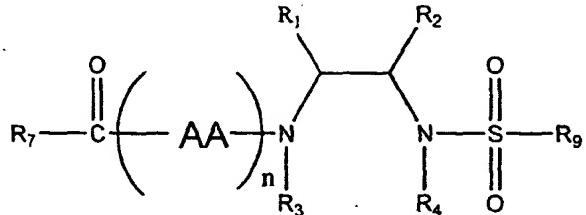
n is from 1 to 10;

PG is a protecting group;

5 AA is an amino acid; and

-SO₂R₉ is a sulfonyl moiety.

8. A cysteine protease inhibitor according to claim 2 having a formula comprising:



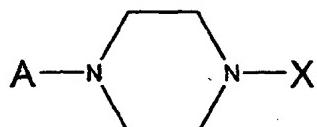
wherein

n is from 1 to 10;

10 AA is an amino acid; and

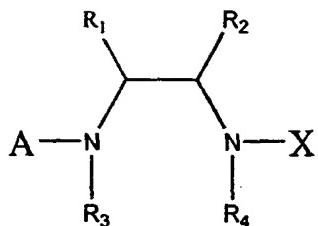
-SO₂R₉ is a sulfonyl moiety.

9. A cysteine protease inhibitor according to claim 2 having a formula comprising:



10. A method of inhibiting a cysteine protease inhibitor comprising reversibly binding a

15 cysteine protease inhibitor having a formula comprising:



wherein

A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl;

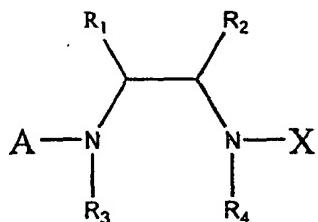
5 R₁ is (a) an amino acid side chain or (b) hydrogen;

R₂ is (a) an amino acid side chain or (b) hydrogen, wherein either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen; and

10 R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene;

wherein the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

15 11. A cysteine protease inhibited by a cysteine protease inhibitor having the formula comprising:



wherein

A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl;

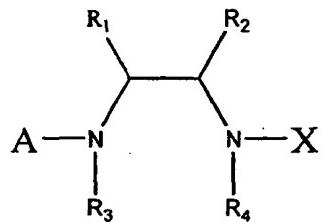
20 R₁ is (a) an amino acid side chain or (b) hydrogen;

R₂ is (a) an amino acid side chain or (b) hydrogen, wherein either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen; and

5 R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene;

wherein the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

12. A method of treating cysteine protease associated disorders comprising administering to a patient a therapeutically effective dose of cysteine protease inhibitor
10 having the formula comprising:



wherein

A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl
15 peptidyl;

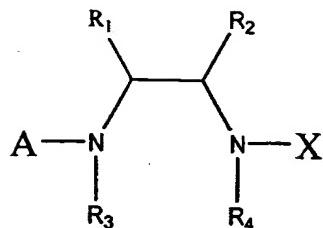
R₁ is (a) an amino acid side chain or (b) hydrogen;

R₂ is (a) an amino acid side chain or (b) hydrogen, wherein either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen; and

20 R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene;

wherein the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

13. A pharmaceutical composition comprising a cysteine protease inhibitor having a
25 formula comprising:



wherein

A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl, alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl;

5

R₁ is (a) an amino acid side chain or (b) hydrogen;

R₂ is (a) an amino acid side chain or (b) hydrogen, wherein either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen; and

10

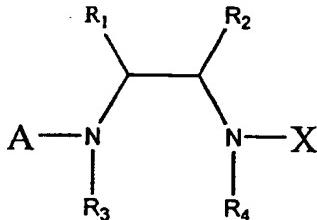
R₃ and R₄ are hydrogen, or are bonded together to form ethylene or substituted ethylene;

wherein the dissociation constant for inhibition, K_i, of a protease with the inhibitor, is no greater than about 100 μM.

14. A method of detecting a cysteine protease in a sample comprising:

15

a) assaying said sample for protease activity using a protease substrate;
 b) assaying for protease activity in the presence of a known concentration of a cysteine protease inhibitor, said inhibitor having a formula comprising:



wherein

A and X are N-substituents selected from the group consisting of acyl, acyl peptidyl,

20

alkyloxycarbonyl, alkyloxycarbonyl peptidyl, sulfonyl, sulfonyl peptidyl, peptidyl, sulfamoyl, sulfamoyl peptidyl, sulfinyl, sulfinyl peptidyl, carbamoyl, and carbamoyl peptidyl;

R₁ is (a) an amino acid side chain or (b) hydrogen;

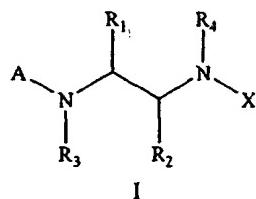
R₂ is (a) an amino acid side chain or (b) hydrogen, wherein either both R₁ and R₂ are hydrogen, or one of R₁ or R₂ is an amino acid side chain and the other one of R₁ and R₂ is hydrogen; and

R_3 and R_4 are hydrogen, or are bonded together to form ethylene or substituted

5 ethylene;

wherein the dissociation constant for inhibition, K_i , of a protease with the inhibitor, is no greater than about 100 μM .

15. A compound of Formula I:

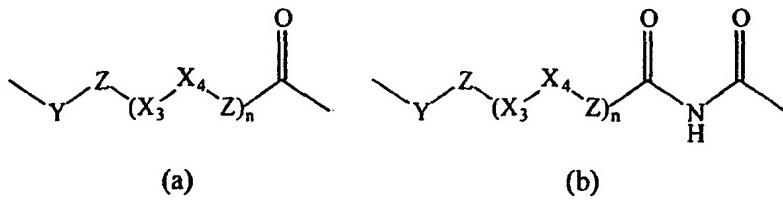


10 in which:

A and X are independently $R_{13}X_{1-}$, wherein R_{13} is hydrogen, alkyloxycarbonylalkanoyl, alkyloxycarbonyl, alkanoyl (optionally substituted with a radical selected from carboxy, alkyloxycarbonyl and heterocycloalkylalkanoylamino), cycloalkylcarbonyl, heterocycloalkylcarbonyl (optionally substituted with a radical selected from hydroxy,

15 alkyl, heterocycloalkyl, alkanoyl, alkyloxycarbonyl, arylalkyloxycarbonyl and heterocycloalkylcarbonyl), arylalkyloxycarbonyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, arylcarbamoyl, arylalkylcarbamoyl, arylalkanoyl, aroyl, alkylsulfonyl, arylsulfonyl, arylalkylsulfonyl, alkylsulfamoyl, dialkylsulfamoyl, arylsulfamoyl, alkylsulfinyl, dialkylaminosulfinyl or arylsulfinyl and X, is a bond or a divalent radical of

20 Formulae (a) or (b):



in which n is 0 to 9; X_3-X_4 represents a linkage selected from $-C(O)NR_{14}-$, $-CH_2NR_{14}-$,
 $-C(O)CH_2-$ and $-NR_{14}C(O)-$; Y is $-CH(R_{14})-$ or $-NR_{14}-$; and Z is $-(CH_2)_2-$, $-C(R_{15})(R_{16})-$ or
 $-N(R_{16})-$; wherein R_{14} is hydrogen or as defined below, R_{15} is hydrogen or methyl and
each R_{16} is independently hydrogen, alkyl (optionally substituted with a radical selected
5 from hydroxy, alkyloxy, amino, alkylamino, dialkylamino, uriedo, alkyluriedo, mercapto,
alkylthio, carboxy, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkylsulfinyl,
alkylsulfonyl, guanidino, $-P(O)(OR_{12})_2$, $-OP(O)(OR_{12})_2$ or $-OP(O)(R_{12})_2$, wherein each
10 R_{12} is independently hydrogen or alkyl, or a protected derivative thereof), cycloalkyl,
cycloalkylalkyl, a group selected from aryl and arylalkyl (which group is optionally
substituted at its aryl ring with one to three radicals selected from hydroxy, amino,
guanidino, halo, optionally halo-substituted alkyl, alkyloxy and aryl, or a protected
15 derivative thereof) or together with an adjacent R_{14} forms a divalent radical selected
from (C_{3-4}) methylene and 1,2-phenylenedimethylene (which radical is optionally
substituted with hydroxy, or a protected derivative thereof, or oxo), with the proviso X
and A are not both hydrogen;
R₁ and R₂ are both hydrogen or one of R₁ or R₂ is cyano, carboxy, alkyloxycarbonyl,
alkanoyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkyloxy(alkyl)carbamoyl,
aminoalkylcarbamoyl, R₁₆, as defined above, or $R_{13}-X_2-$, wherein R₁₃ is as defined
above and X₂ is a divalent radical of Formulae (a) or (b), as defined above; and
20 R₃ and R₄ together form optionally substituted ethylene or are independently R₁₄, as
defined above; and the pharmaceutically acceptable salts, individual isomers and
mixtures of isomers thereof.

16. The compound of Claim 15 in which A is $R_{13}-X_1-$, wherein R₁₃ is hydrogen,
alkyloxycarbonylalkanoyl of overall 3 to 10 carbon atoms, (C_{1-9}) alkoxycarbonyl,
25 (C_{2-10}) alkanoyl (optionally substituted with a radical selected from carboxy,
 (C_{1-9}) alkyloxycarbonyl and hetero (C_{4-8}) cycloalkyl (C_{2-10}) alkanoylamino),
 (C_{4-8}) cycloalkylcarbonyl, hetero (C_{4-8}) cycloalkylcarbonyl (optionally substituted with a
radical selected from hydroxy, (C_{1-5}) alkyl, hetero (C_{4-8}) cycloalkyl, (C_{1-5}) alkanoyl,
30 (C_{1-5}) alkyloxycarbonyl, (C_{6-10}) aryl (C_{1-5}) alkyloxycarbonyl and
hetero (C_{4-8}) cycloalkylcarbonyl), (C_{6-10}) aryl (C_{1-5}) alkyloxycarbonyl, carbamoyl,
 (C_{1-5}) alkylcarbamoyl, di (C_{1-5}) alkylcarbamoyl, (C_{6-10}) arylcarbamoyl,
 (C_{6-10}) aryl (C_{1-5}) alkylcarbamoyl, (C_{6-10}) aryl (C_{1-5}) alkanoyl, (C_{7-11}) aroyl, (C_{1-10}) alkylsulfonyl,
di (C_{1-5}) alkylsulfamoyl, (C_{6-10}) arylsulfonyl, (C_{6-10}) aryl (C_{1-5}) alkylsulfonyl or
hetero (C_{4-8}) arylsulfonyl; and X₁ is a divalent radical of Formula (a), wherein n is 0 to 5;

X₁-X₂ represents a linkag selected from -C(O)NR₁₄-; Y is -N(R₁₄)-; Z is -CH(R₁₆)-; R₁₆ is hydrogen or as defined below; and R⁸ is (C₃₋₇)cycloalkyl, (C₃₋₇)cycloalkyl(C₁₋₅)alkyl, pyridyl, thienyl, furyl, imidazolyl, indolyl, pyridyl(C₁₋₆)alkyl, thienyl(C₁₋₆)alkyl, furyl(C₁₋₆)alkyl, imidazolyl(C₁₋₆)alkyl, indolyl(C₁₋₆)alkyl, (C₁₋₅)alkyl (optionally substituted with a radical selected from mercapto, carboxy, amino, methylthio, methylsulfonyl, carbamoyl, dimethylcarbamoyl, guanidino and hydroxy, or a protected derivative thereof), a group selected from phenyl, naphthyl, phenyl(C₁₋₆)alkyl, naphthyl(C₁₋₆)alkyl, (which group is optionally substituted at its aryl ring with one to three radicals selected from amino, hydroxy, chloro, bromo, fluoro, iodo, methyl, trifluoromethyl, methoxy and phenyl, or a protected derivative thereof) or together with an adjacent R₁₄ forms a divalent radical selected from (C₃₋₄)methylene and 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo); X is -S(O)₂R₉, wherein R₉ is (C₁₋₅)alkyl (optionally substituted with one or two radicals selected from amino, chloro, bromo, fluoro, hydroxy and methoxy, or a protected derivative thereof), perhalo(C₁₋₅)alkylsulfonyl, (C₃₋₇)cycloalkyl, (C₃₋₇)cycloalkyl(C₁₋₅)alkyl or a group selected from phenyl, pentafluorophenyl, naphthyl and phenyl(C₁₋₆)alkyl (which group is optionally substituted at its aryl ring with one to two radicals selected from amino, chloro, bromo, fluoro, hydroxy, methoxy and optionally halo-substituted methyl, or a protected derivative thereof);

5 R₁ is hydrogen and R₂ is R₁₆, as defined above; and

10 R₃ and R₄ are each hydrogen or together form optionally substituted ethylene.

15

20

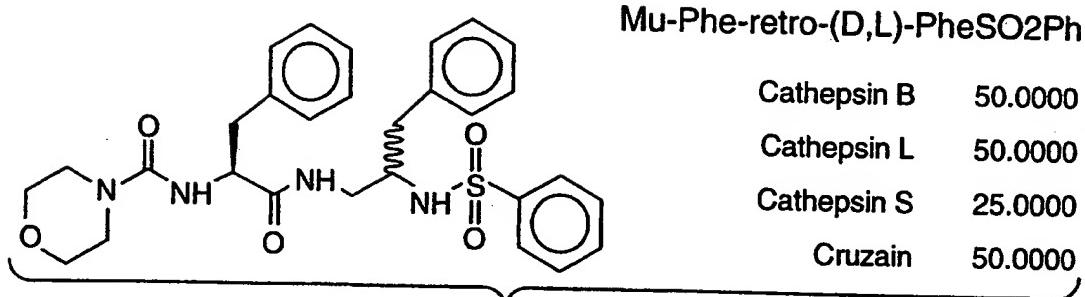
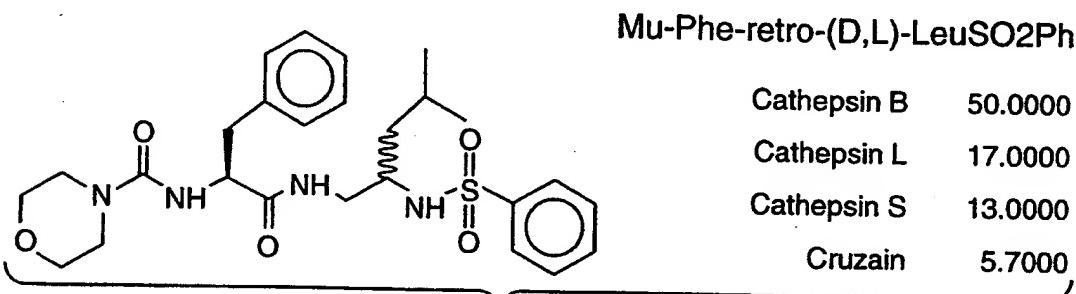
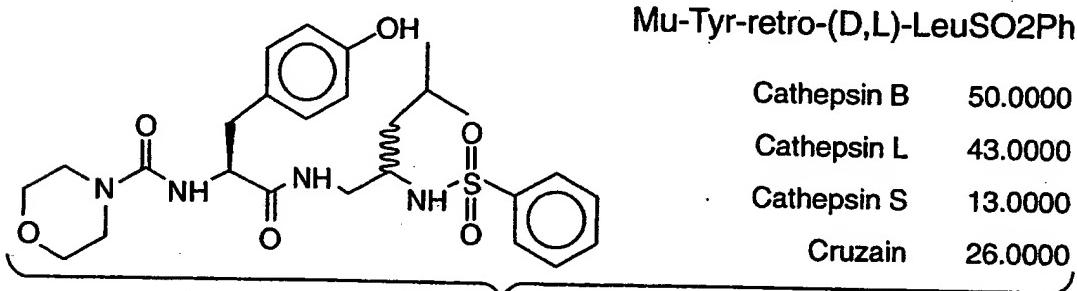
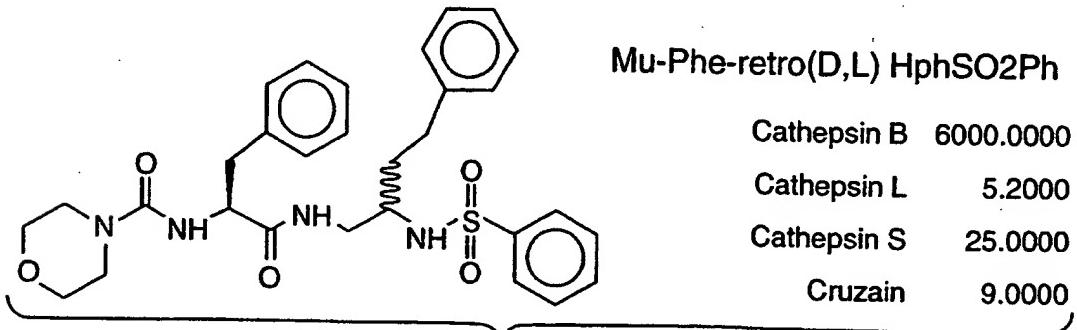
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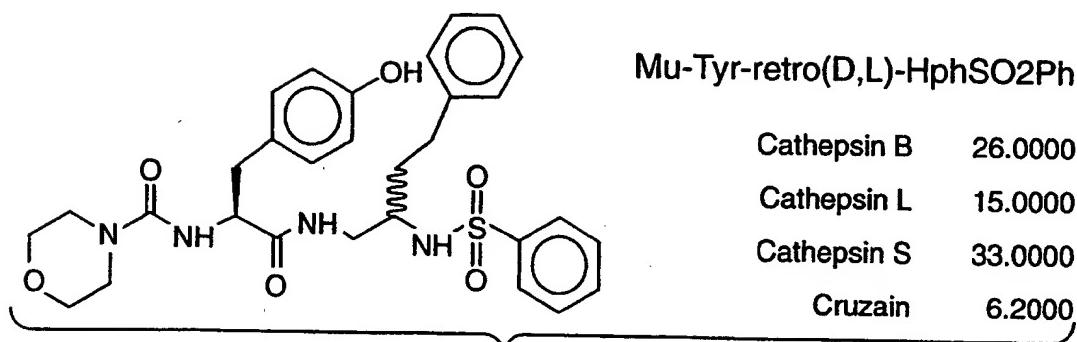
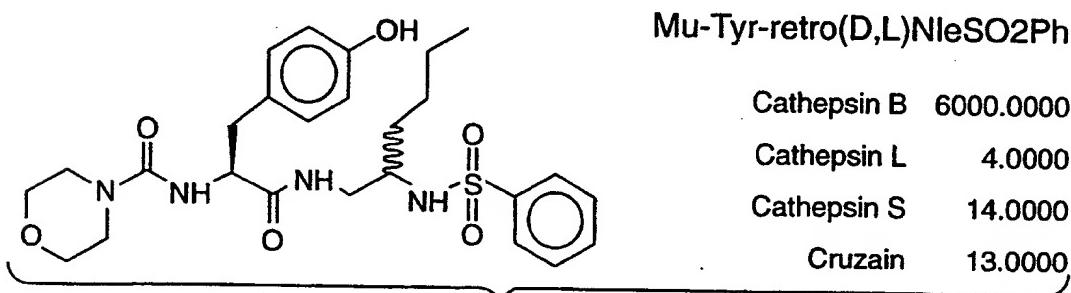
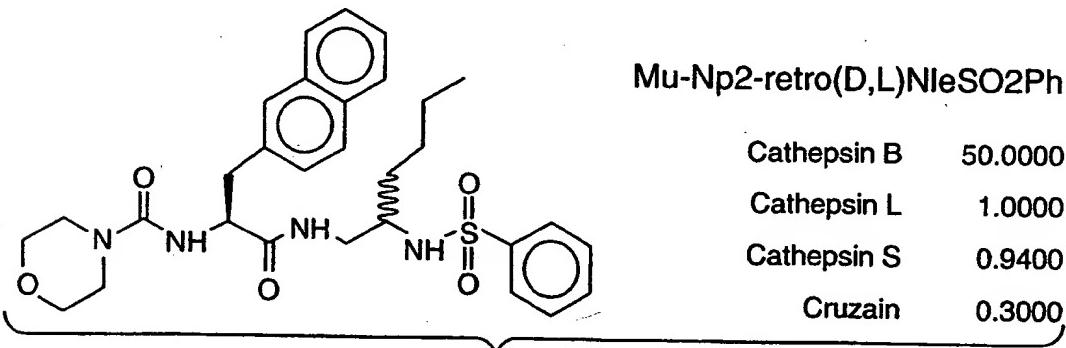
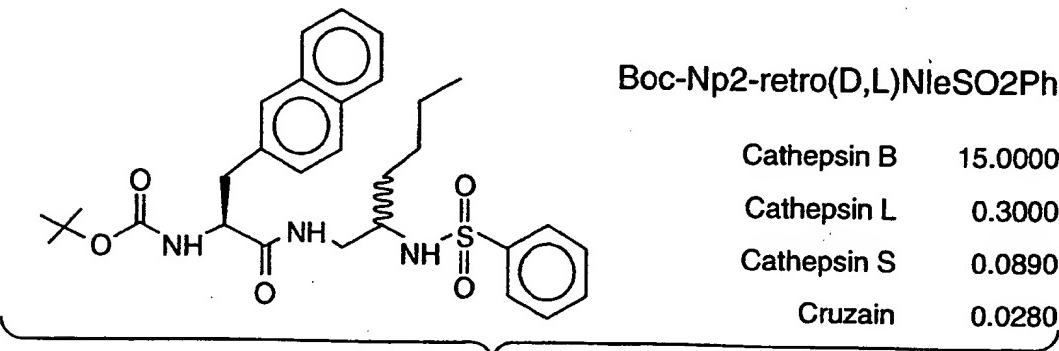
17. The compound of Claim 16 wherein n is 0 to 2; R₂ is R₁₆, as defined below; R₃ and R₄ together form optionally substituted ethylene; R₁₃ is hydrogen, (C₄₋₈)alkoxycarbonyl, (C₂₋₆)alkanoyl (optionally substituted with a radical selected from carboxy, (C₁₋₅)alkyloxycarbonyl and hetero(C₄₋₈)cycloalkyl(C₄₋₈)alkanoylamino), -C(O)NR₁₀R₁₁, wherein R₁₀ and R₁₁ together form aza(C₂₋₆)methylene, oxa(C₂₋₆)methylene or (C₃₋₇)methylene, (C₄₋₈)cycloalkylcarbonyl, benzyloxycarbonyl, acetyl, benzoyl or dimethylsulfamoyl; R₈ is (C₅₋₆)cycloalkyl, (C₅₋₆)cycloalkylmethyl, 3-pyridyl, 2-thienyl, 2-furyl, 4-imidazolyl, 3-indolyl, 3-pyridylmethyl, 2-thienylmethyl, 2-furylmethyl, 4-imidazolylmethyl, 3-indolylmethyl, methoxy, acetoxy, (C₁₋₅)alkyl (optionally substituted with a radical selected from mercapto, carboxy, amino, methylthio, methylsulfonyl, carbamoyl, dimethylcarbamoyl, guanidino and hydroxy, or a protected derivative thereof), a group selected from phenyl, 1-naphthyl, 2-naphthyl, benzyl, 1-naphthylmethyl, 2-naphthylmethyl and 2-phenylethyl (which group is optionally

- substituted at its aryl ring with one radical selected from hydroxy, amino, chloro, bromo and fluoro, or a protected form thereof) or together with an adjacent R₁₄ forms a
5 divalent radical selected from (C₃₋₄)methylene and 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo); and R₉ is (C₁₋₅)alkyl (optionally substituted with one or two radicals selected from amino, chloro, bromo, fluoro and hydroxy, or a protected derivative thereof), perfluoro(C₁₋₅)alkyl, (C₅₋₆)cycloalkyl, (C₅₋₆)cycloalkylmethyl or a group selected from phenyl, naphthyl and benzyl (which group is optionally substituted with one radical selected from amino hydroxy, chloro, bromo or fluoro, or a protected derivative
10 thereof).
18. The compound of Claim 17 in which n is 0 to 1; R₂ is butyl, 2-phenylethyl, 2-methylsulfonylethyl, 2-*tert*-butoxycarbonylethyl, 2-*tert*-butoxycarbonylmethyl, 4-*tert*-butoxycarbonylaminobutyl, 4-benzoylaminobutyl or benzyloxymethyl; R₁₃ is hydrogen, *tert*-butoxycarbonyl, benzyloxycarbonyl, acetyl, 3-carboxypropionyl,
15 3-methoxycarbonylpropionyl, biotinylaminohexanoyl, phenylacetyl, benzoyl, dimethylsulfamoyl, benzylsulfonyl, 1-piperizinylcarbonyl, 4-methyl-1-piperazinylcarbonyl or 4-morpholinylcarbonyl; R₁₆ is 3-pyridylmethyl, 2-thienylmethyl, 2-furylmethyl, 4-imidazolylmethyl, 3-indolylmethyl, (C₁₋₅)alkyl (optionally substituted with a radical selected from mercapto, carboxy, amino, methylthio, methylsulfonyl, carbamoyl, dimethylcarbamoyl, guanidino and hydroxy, or a protected derivative thereof), a group selected from benzyl, 1-naphthylmethyl, 2-naphthylmethyl and 2-phenylethyl (which group is optionally substituted at its aryl ring with one radical selected from hydroxy, amino, chloro, bromo and fluoro, or a protected form thereof) or together with an adjacent R₃ or R₁₃ forms a divalent radical selected from (C₃₋₄)methylene and
20 1,2-phenylenedimethylene (which radical is optionally substituted with hydroxy, or a protected derivative thereof, or oxo); and R₉ is methyl, trifluoromethyl, optionally substituted phenyl, 2-naphthyl or 2-phenylethyl.
19. The compound of Claim 18 in which n is 0; R₂ is butyl, 2-phenylethyl or 2-methylsulfonylethyl; R₁₃ is hydrogen, *tert*-butoxycarbonyl, benzyloxycarbonyl,
25 biotinylaminohexanoyl, benzoyl, 1-piperizinylcarbonyl, 4-methyl-1-piperazinylcarbonyl or 4-morpholinylcarbonyl; R₁₆ is (C₁₋₅)alkyl, optionally substituted benzyl, 1-naphthylmethyl, 2-naphthylmethyl, 3-pyridinylmethyl or 2-methylsulfonylethyl; and R₉ is phenyl, 1-naphthyl or 2-phenylethyl.
30

1 / 11

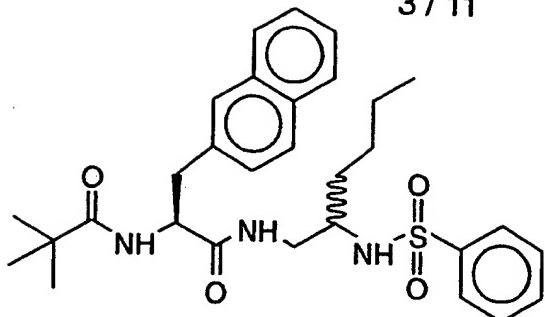
**FIG._1A****FIG._1B****FIG._1C****FIG._1D**

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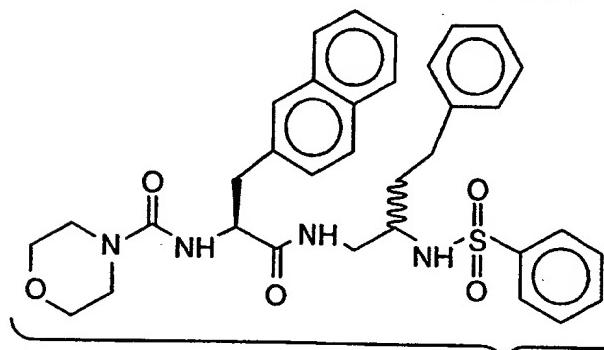
**FIG._1E****FIG._1F****FIG._1G****FIG._1H**

SUBSTITUTE SHEET (RULE 26)

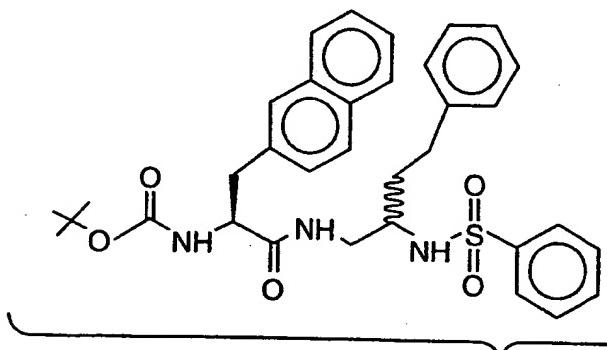
3 / 11

Piv-Np2-retro(D,L)NleSO₂Ph

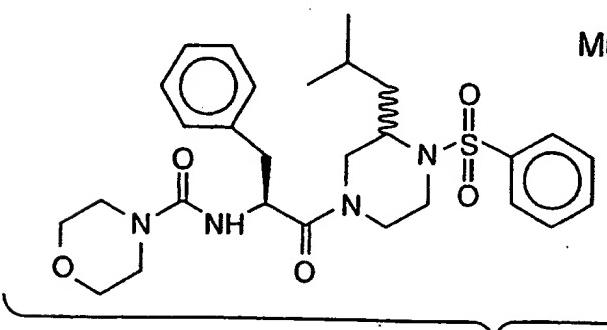
Cathepsin B	10.0000
Cathepsin L	0.2000
Cathepsin S	0.2300
Cruzain	0.4200

FIG._1I

Cathepsin B	50.0000
Cathepsin L	1.8000
Cathepsin S	2.2000
Cruzain	1.0000

FIG._1J

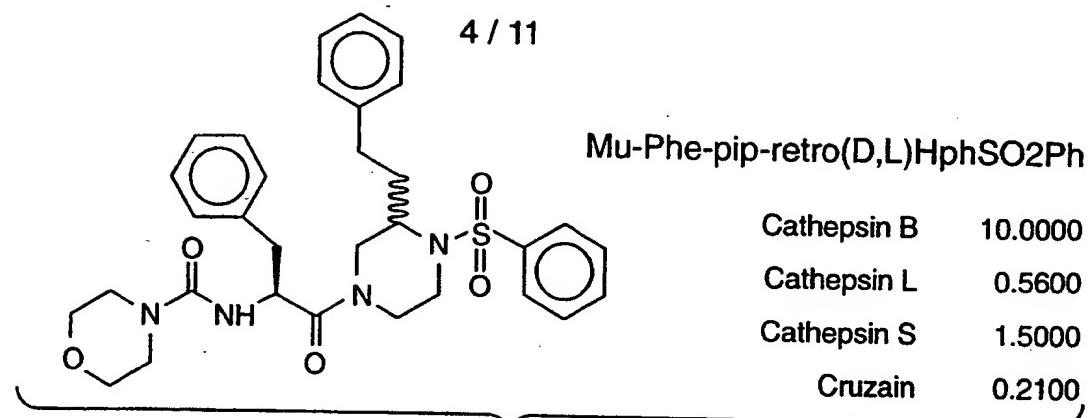
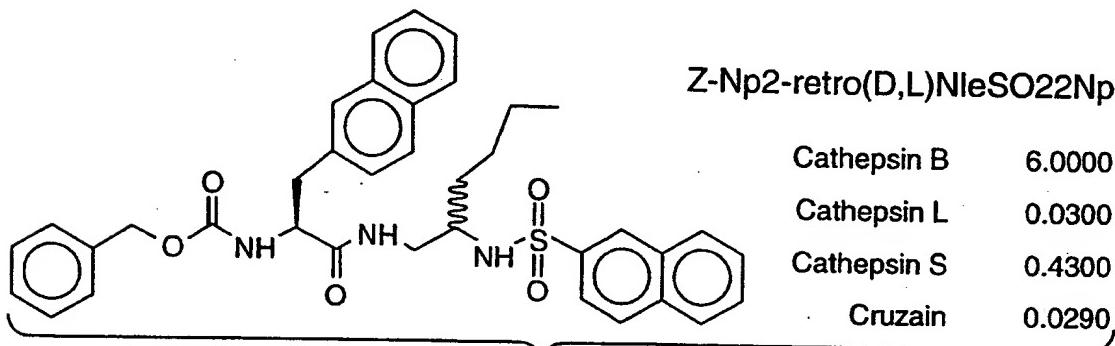
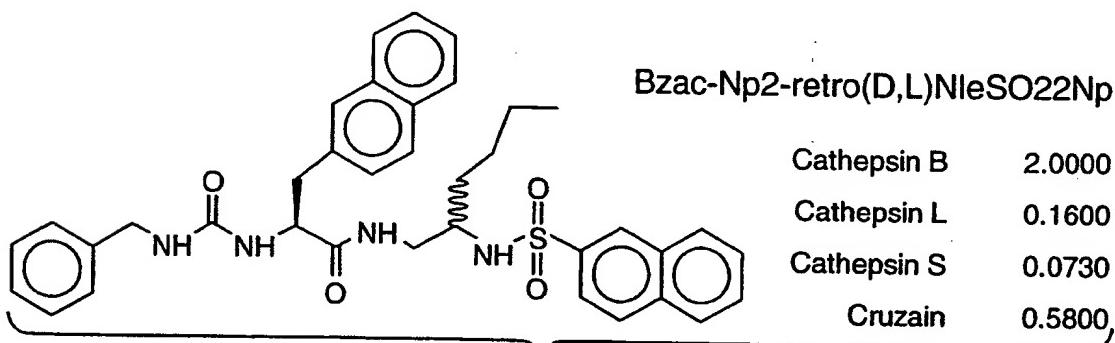
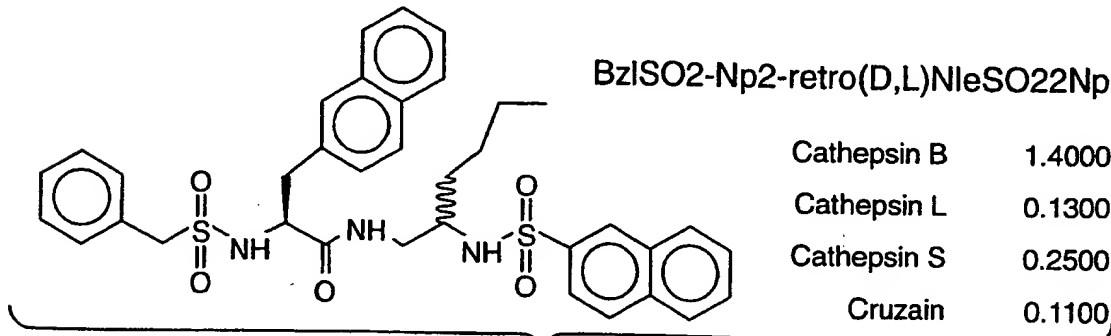
Cathepsin B	5.3000
Cathepsin L	1.1000
Cathepsin S	2.0000
Cruzain	0.3000

FIG._1K

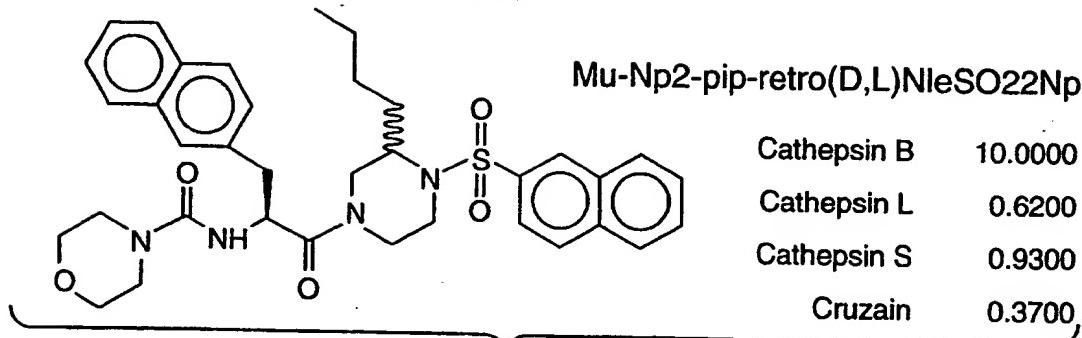
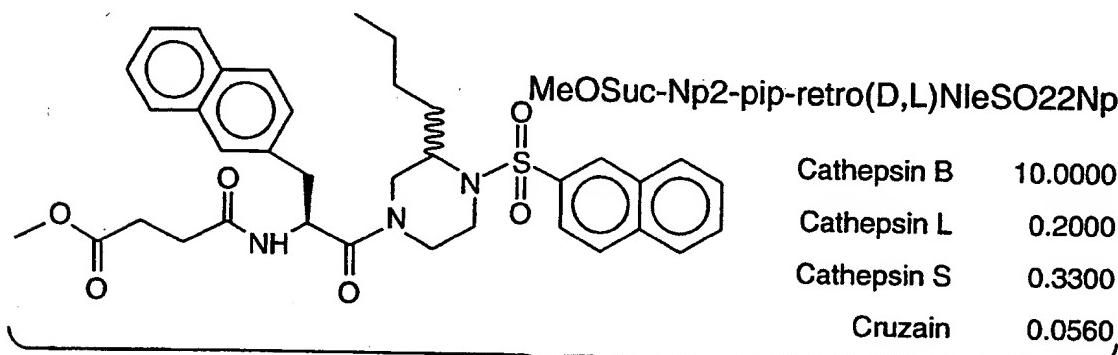
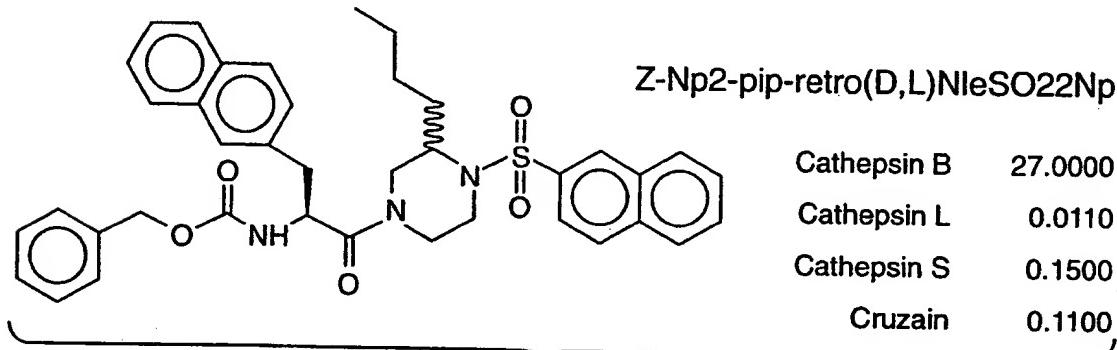
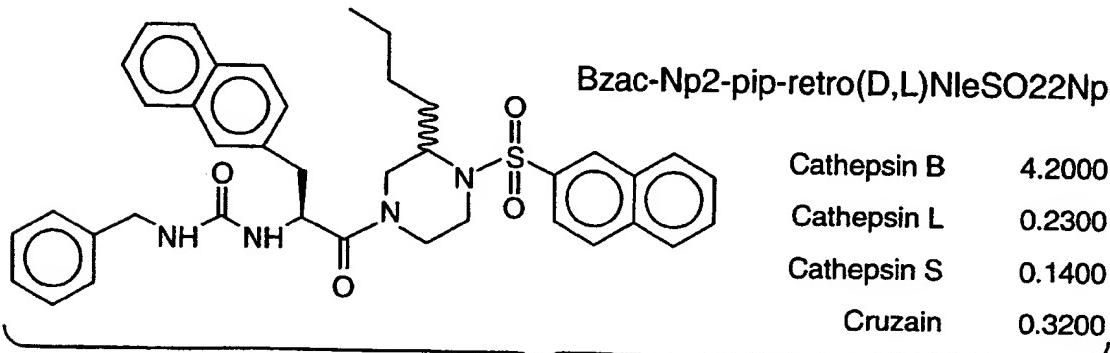
Cathepsin B	50.0000
Cathepsin L	13.0000
Cathepsin S	51.0000
Cruzain	75.0000

FIG._1L

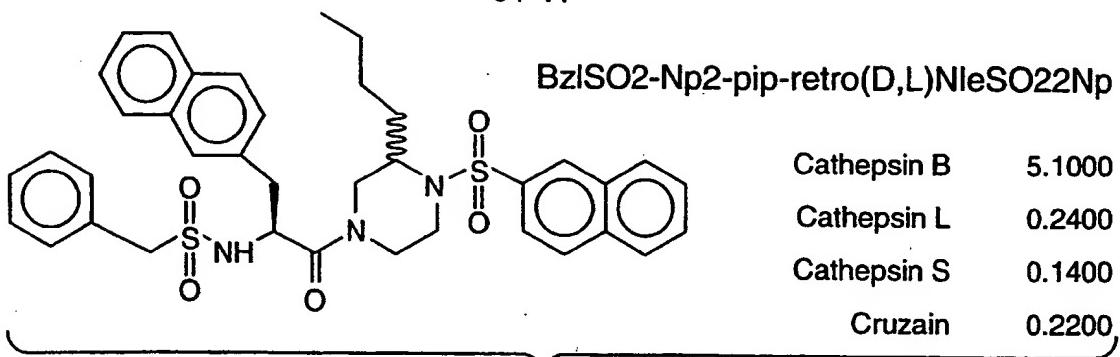
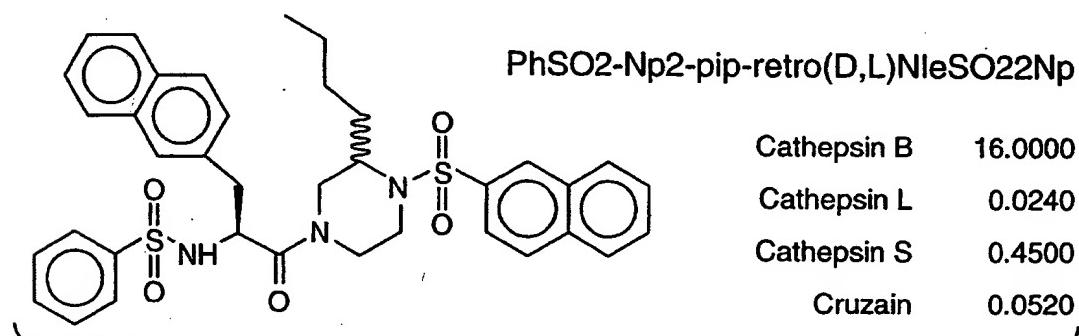
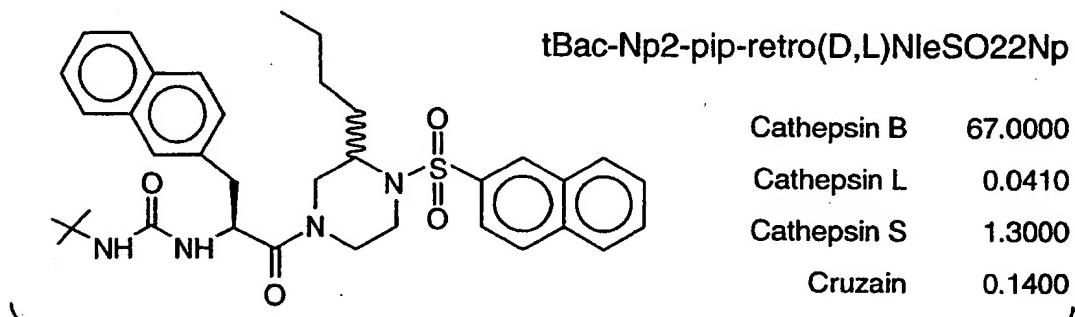
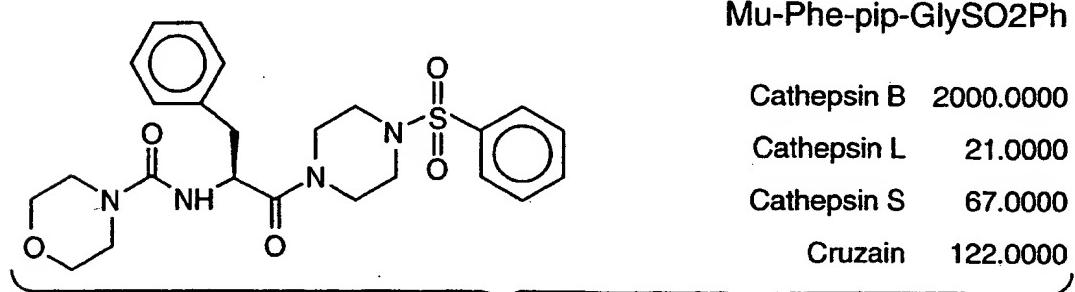
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**FIG._1M****FIG._1N****FIG._1O****FIG._1P**

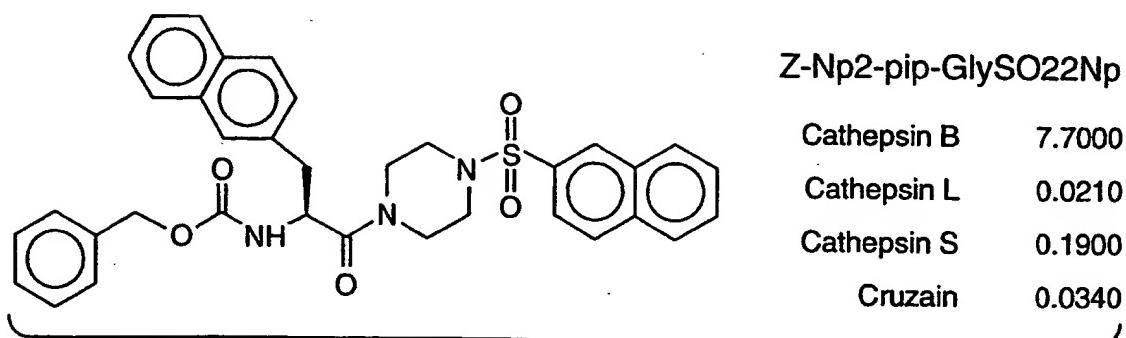
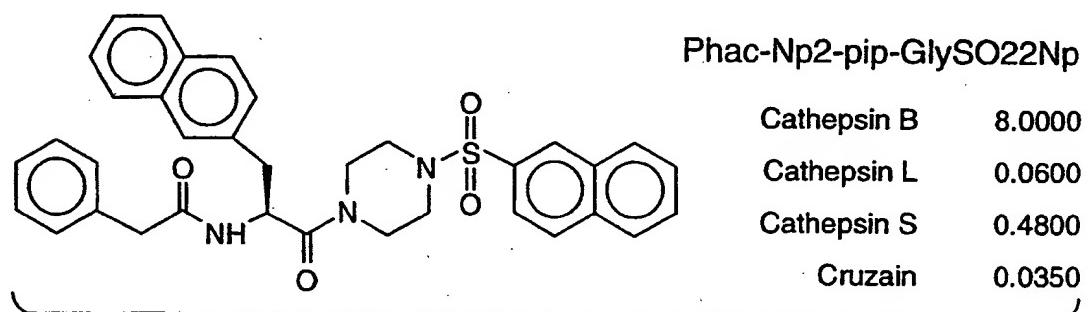
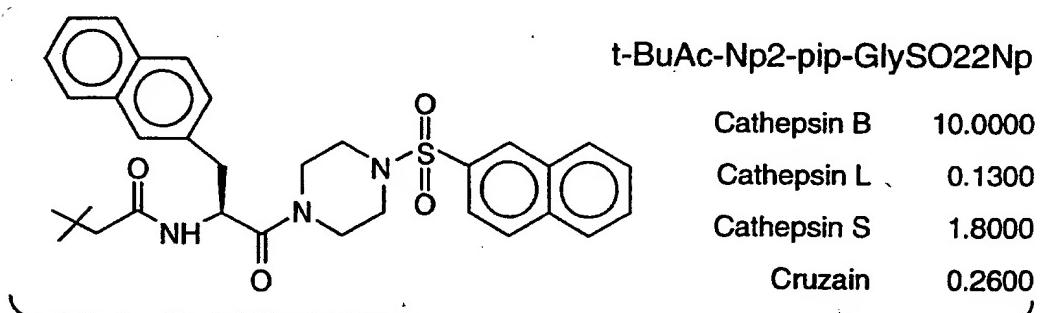
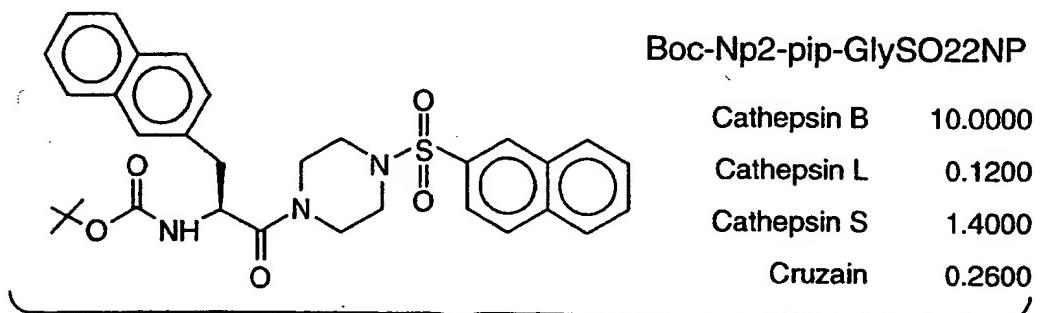
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**FIG._1Q****FIG._1R****FIG._1S****FIG._1T**

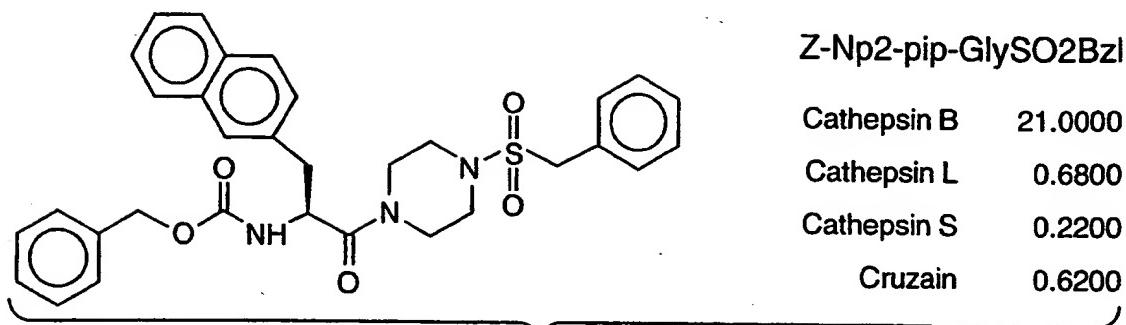
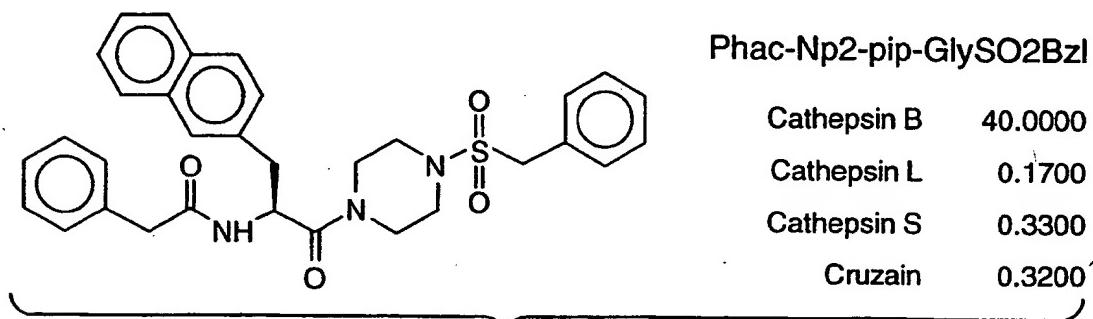
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**FIG._1U****FIG._1V****FIG._1W****FIG._1X**

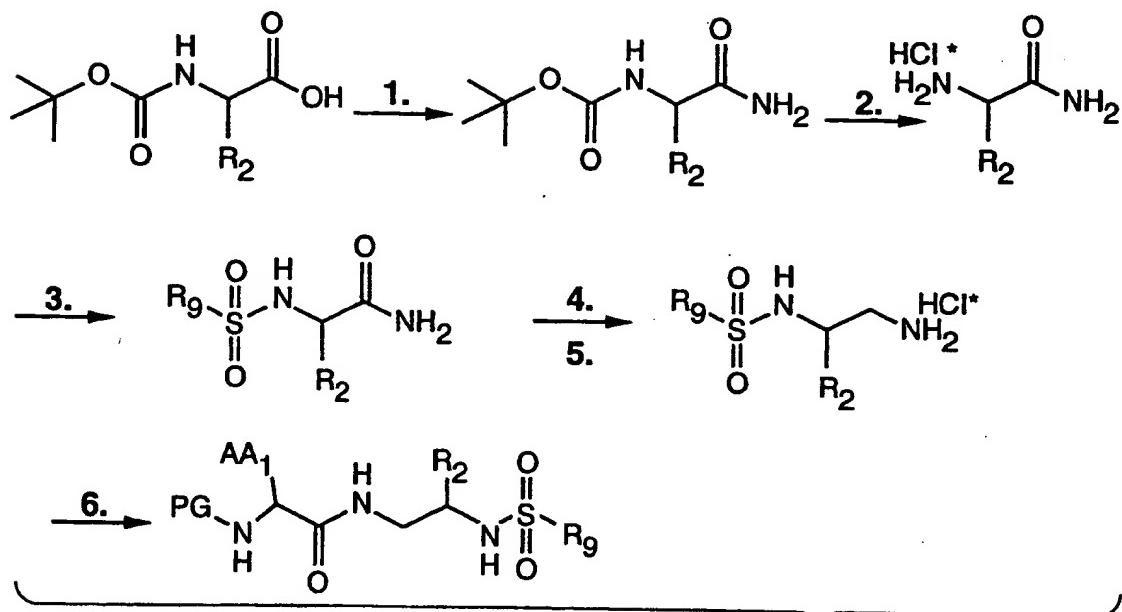
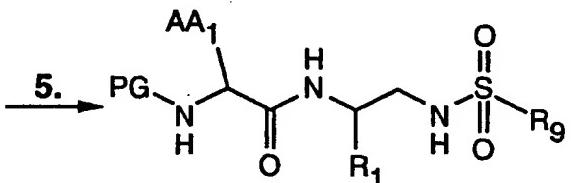
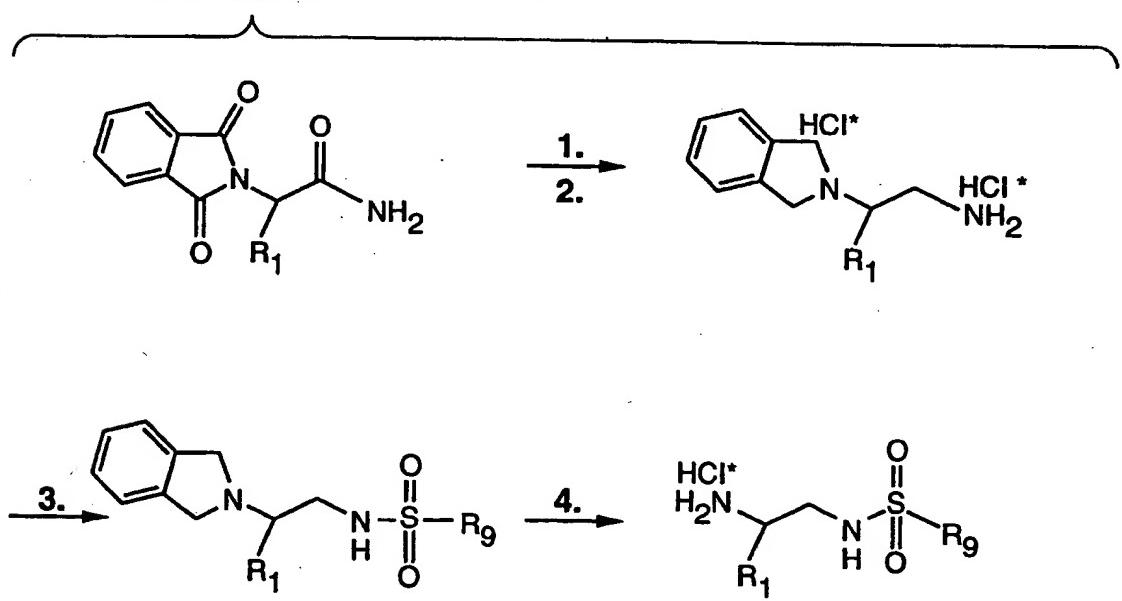
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**FIG._1Y****FIG._1Z****FIG._1AA****FIG._1BB**
SUBSTITUTE SHEET (RULE 26)

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**FIG._1CC****FIG._1DD**

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**FIG. 2****FIG. 3****SUBSTITUTE SHEET (RULE 26)**

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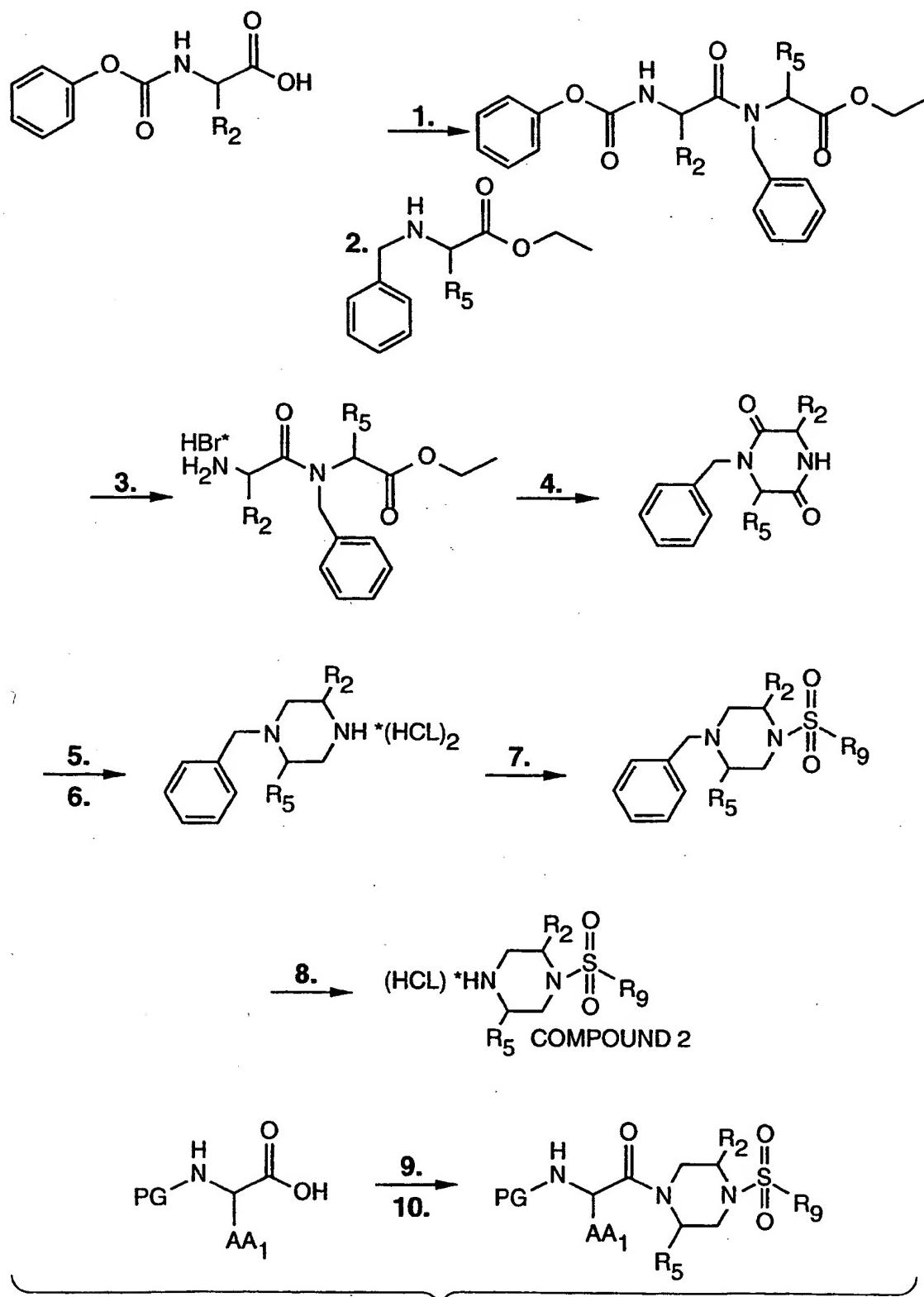


FIG._4
SUBSTITUTE SHEET (RULE 26)

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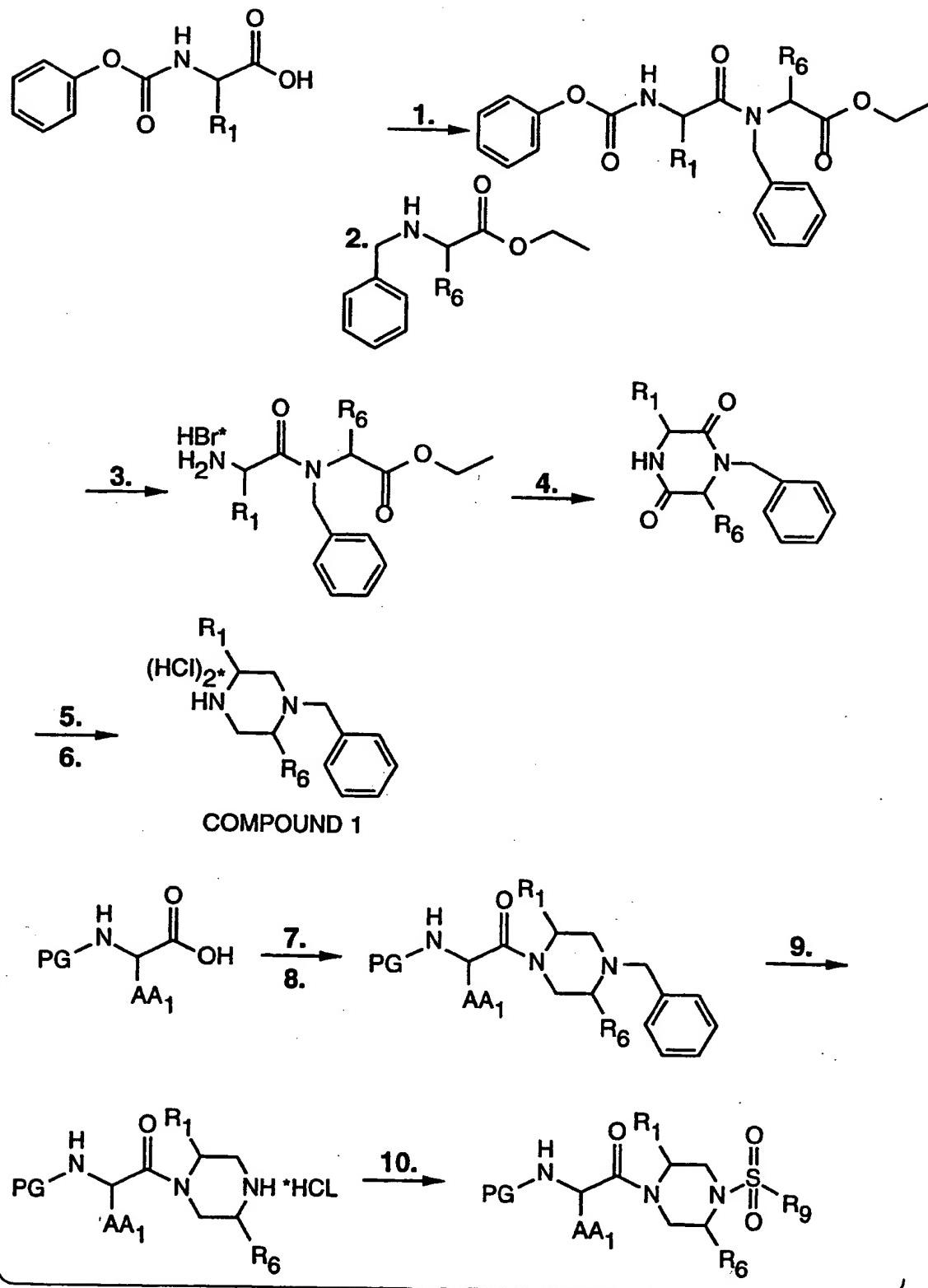


FIG._5
SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/08559

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 6	C07K5/00	C07D295/26	C07D295/215	C07C311/15	A61K38/04
A61K31/18					

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07D C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 18185 (PENTAPHARM AG) 18 August 1994	1-9, 15-19
Y	* claims; examples *	1-19

Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 88365136 XP002014649 & JP,A,63 275 575 (NIPPON CHEMIFAR) , 14 November 1988 see abstract	1-19

	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

1

Date of the actual completion of the international search

30 September 1996

Date of mailing of the international search report

22.10.96

Name and mailing address of the ISA

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Fax (+ 31-70) 340-3016

Authorized officer

Hermann, R

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/08559

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOMEDICAL RESEARCH, vol. 4, no. 1, 1983, pages 121-124, XP000602773 HARA, K. & TAKAHASHI, K.: "Inhibition of calcium-activated neutral protease of monkey cardiac muscle by eposuccinic acid derivatives" * page 121 * * compounds 17 and 18 *	11
Y	-----	1-19

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/08559

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 1-5, 9-19 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see continuation-sheet

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/08559

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9418185	18-08-94	AU-A-	5878194	29-08-94
		CA-A-	2133761	18-08-94
		CZ-A-	9402459	18-10-95
		EP-A-	0635008	25-01-95
		HU-A-	68042	22-03-95
		JP-T-	7509731	26-10-95